brain sciences

Volume 2

# Advances in Autism Research

Edited by

Antonio Narzisi

Printed Edition of the Special Issue Published in *Brain Sciences*

www.mdpi.com/journal/brainsci

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## Volume 2

Editor

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This is a reprint of articles from the Special Issue published online in the open access journal *Actuators* (ISSN 2076-0825) (available at: https://www.mdpi.com/journal/brainsci/special_issues/Advance_Autism_Research).

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

LastName, A.A.; LastName, B.B.; LastName, C.C. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

**Volume 2**
**ISBN 978-3-0365-0202-1 (Hbk)**
**ISBN 978-3-0365-0203-8 (PDF)**

**Volume 1-2**
**ISBN 978-3-0365-0204-5 (Hbk)**
**ISBN 978-3-0365-0205-2 (PDF)**

Cover image courtesy of pixabay.com user John Silver.

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# About the Editor

**Antonio Narzisi** is Lead Psychologist at the Department of Child Psychiatry and Psychopharmacology (Head: Gabriele Masi) at the IRCCS Stella Maris Foundation sited in Calambrone (Pisa) and Adjunct Professor at University of Pisa. He was born in Avola in the province of Siracusa (Sicily). Narzisi studied in Rome where he graduated in Psychology at La Sapienza University (Advisor: Massimo Ammaniti). He then moved to Tuscany, where he obtained his PhD in Developmental Neurosciences at the University of Pisa (Advisor: Filippo Muratori). He was certified as Psychotherapist at the Gestalt Institute HCC-Italy, Milan (Advisor: Margherita Spagnuolo Lobb). Narzisi is a recognized expert in the field of Autism Spectrum Disorder (ASD) and is specialized in evidence-based treatment models and gold standard diagnostic protocols. His clinical and research activity is focused on the diagnosis of Autism Spectrum Disorder, treatment outcomes, and the application of new technologies in the clinical and daily living settings. During the years, Narzisi was an active participant in the COST Action BM1004 (Enhancing the scientific study of early autism: A network to improve research, services, and outcomes) and has Full Membership at INSAR – International Society for Autism Research. In 2012, he was a visiting scientist at the Institute of Brain, Behavior and Mental Health, University of Manchester (UK) (Head: Jonathan Green). He has served as co-investigator in several research projects funded by the European Commission and Italian Ministry of Health. Narzisi is the author of numerous publications in recognized international journals. Narzisi has presented the results of his research in international conferences (USA, Canada, UK, France, Malta, Belgium, Morocco, and Italy). He teaches "Autism Best Practices" in several Italian universities, Masters and Clinical Institute that are accredited by the Italian National Health Service. He was recently nominated Local Coordinator (for IRCCS Stella Maris Foundation) of the bio-behavioral bank of ITAN (Italian Autism Network).

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Article

# Preconception Risk Factors for Autism Spectrum Disorder—A Pilot Study

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Received: 13 April 2020; Accepted: 12 May 2020; Published: 14 May 2020

**Abstract:** Autism spectrum disorder (ASD) is a neurodevelopmental disorder of multifactorial etiology. Preconception risk factors are still poorly understood. A survey on preconception risk factors for ASD was conducted among parents of 121 ASD patients aged 3–12 years and parents of 100 healthy children aged 3–12 years. The exclusion criteria were as follows: the presence of associated problems such as intellectual disability, epilepsy or other genetic and neurological diseases. Thirteen parameters were considered, a few among which were conception problems, conception with assisted reproductive techniques, the use and duration of oral contraception, the number of previous pregnancies and miscarriages, time since the previous pregnancy (in months), the history of mental illness in the family (including ASD), other chronic diseases in the mother or father and maternal and paternal treatment in specialist outpatient clinics. Three factors statistically significantly increased the risk of developing ASD: mental illness in the mother/mother's family (35.54% vs. 16.0%, $p = 0.0002$), maternal thyroid disease (16.67% vs. 5.0%, $p = 0.009$) and maternal oral contraception (46.28% vs. 29.0%, $p = 0.01$). Children of mothers with thyroid disorders or with mental illness in relatives should be closely monitored for ASD. Further studies are warranted to assess a potential effect of oral contraception on the development of offspring.

**Keywords:** autism; preconception risk factor

## 1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder defined by abnormalities in communication and social interaction, delayed development and repetitive, stereotypical activities [1]. The prevalence of ASD has increased in recent years. It is estimated at 1.34% among 4-year-old children in the USA [2]. The etiology of the disorder is not fully understood. It is assumed that the etiology is most likely multifactorial and the phenotypic expression is influenced by genetic conditions and environmental factors. Some recent studies have demonstrated that the impact of environmental factors can be as high as 40–50% [3–5]. These are of great importance because while genetic factors are not currently modifiable, the elimination of potential environmental risk factors could reduce the risk of the manifestation of ASD. The mechanisms of the association between environmental factors and ASD are debated but might include non-causative association (including confounding), gene-related effect, oxidative stress, inflammation, hypoxia/ischemia, endocrine disruption, neurotransmitter alterations, and interference with the signaling pathways [6]. Numerous studies on pregnancy risk factors

have been conducted. Additionally, studies have described different parameters influencing fetal neurodevelopment and, consequently, the development of features typical of the ASD phenotype. However, the influence of factors affecting the father and mother before pregnancy is still poorly understood. To date, several studies have shown that maternal overweight and obesity statistically significantly increase the risk of developing ASD in offspring [7–12]. In addition, maternal opioid use before pregnancy is an independent risk factor for the development of ASD [13]. In turn, the animal model study showed a positive correlation between preconceptional stressful experiences and the occurrence of an ASD-like phenotype in male offspring [14]. Inconclusive results were obtained in relation to the effect of preconception supplementation with vitamins and folic acid [15–17].

The aim of our study was to analyze 13 potential preconception maternal and paternal risk factors for ASD in offspring.

## 2. Material and Methods

### 2.1. Participants

The study group (group 1) consisted of 121 Caucasian children with autism and their biological parents from Silesia (southwestern region of Poland) treated in Katowice or Gliwice (Department of Pediatric Neurology, Child Development Support Center and Psychiatric Daily Ward for Children and Adolescents). The diagnosis was established by a psychiatrist using ADOS-2 (Autism Diagnosis Observation Schedule) as the gold standard observational instrument [18]. The inclusion criteria were as follows: 3–12 years of age and meeting the criteria for ASD. In order to obtain a homogeneous group of patients, which could be defined as the "pure autism group", strict exclusion criteria have been applied, including the occurrence of related problems such as intellectual disability, epilepsy and other genetic and neurological diseases.

The reference group (group 2) included 100 Caucasian children with no symptoms of ASD and their biological parents from the same region of Poland. Participants were recruited from primary schools. The inclusion criteria were as follows: 3–12 years of age and the absence of ASD. The exclusion criteria were established as in group 1, i.e., the simultaneous occurrence of an intellectual disability in a child, epilepsy and other genetic and neurological comorbidities.

### 2.2. Methods

The survey was conducted in 2016–2017 among parents of children in both groups. The questionnaire was completed by experienced physicians based on the information obtained from the parent. Participants were informed that their participation in the study was voluntary and that they could withdraw without consequences. The questionnaire used closed questions, while parents were allowed to use the child's health records. The survey included 13 potential preconception risk factors for ASD in offspring. These factors included conception problems, conception using assisted reproductive techniques, the use and duration of oral contraception, the number of previous pregnancies and miscarriages, time since the previous pregnancy (in months), the history of mental illness in parents and relatives (including ASD) and other chronic diseases in the mother or father (including thyroid disease, cardiovascular disease, ophthalmic disease, and arterial hypertension, epilepsy or diabetes) that occurred before pregnancy, from which the child with ASD was born. Separately, a question was asked about diseases during the pregnancy period (including hypothyroidism). The diseases which rarely occurred in parents were included in the group termed "other".

The study was approved by the Ethical Committee of Medical University of Silesia, and approval code No.: KNW/0022/KB1/27/I/15.

### 2.3. Statistical Analyses

The statistical analysis was performed using STATISTICA 10 PL (StatSoft). Comparisons of the distributions of the prevalence of the analyzed risk factors in both groups were performed using

Fisher's exact test. To compare the time interval between the previous pregnancy and the pregnancy from which the child with ASD was born, the U Mann–Whitney was used. The relative risk ratio (RR) and its 95% confidence interval were calculated and its significance was verified for the factors which reached a statistical significance.

## 3. Results

The detailed demographic data on children and their parents are presented in Table 1. Based on the statistical analysis, there was no significant difference in the age and sex between the study and control groups ($p = 0.20$), however, there were some differences in education.

**Table 1.** Demographic data of the study and reference groups.

| Factor | Category | Study Group $n = 121$ (100%) | Reference Group $n = 100$ (100%) | Significance Level |
|---|---|---|---|---|
| children's sex | male<br>female | 105 (86.78%)<br>16 (13.22%) | 85 (85.0%)<br>15 (15.0%) | $p = 0.70$ |
| children's age | 2–7 years<br>8–12 years | 64 (52.89%)<br>57 (47.11) | 64 (64.0%)<br>36 (36.0%) | $p = 0.10$ |
| mother's age at the conception | | $n = 120$ | $n = 98$ | |
| | ≤35 years<br>>35 years | 112 (93.33%)<br>8 (6.67%) | 91 (92.86%)<br>7 (7.14%) | $p = 0.55$ |
| mother's education | | $n = 119$ | $n = 90$ | |
| | higher<br>secondary *<br>primary * | 73 (61.34%)<br>42 (35.29%)<br>4 (3.36%) | 70 (77.78%)<br>19 (21.11%)<br>1 (1.11%) | **$p = 0.02$** |
| father's age at the conception | | $n = 119$ | $n = 97$ | |
| | ≤35 years<br>>35 years | 103 (86.55%)<br>16 (13.45%) | 75 (77.32%)<br>22 (22.68%) | $p = 0.11$ |
| father's education | | $n = 118$ | $n = 89$ | |
| | higher<br>secondary *<br>primary * | 51 (43.22%)<br>50 (42.37%)<br>17 (14.41%) | 54 (60.67%)<br>33 (37.08%)<br>2 (2.25%) | **$p = 0.02$** |

* counted together for the statistical analysis; Statistically significant figures are marked in bold.

The statistics on the responses to questions on the potential preconception risk factors are included in Tables 2–4. Oral contraception was statistically significantly more often used by mothers from group 1 compared with mothers from group 2 (56/121 (46.28%) vs.29/100 (29.0%); $p = 0.01$), while the duration of contraception was insignificant.

A correlation between the occurrence of mental illness in the mother and/or mother's family and ASD in the child was confirmed (43/121 (35.54%) in group 1 vs. 16/100 (13.0%) in group 2; $p = 0.0002$). Autism spectrum disorder included 8/121 (6.61%) relatives from group 1 and 3/100 (3.0%) relatives from group 2. In turn, mental illness in the father and/or father's family was found to be insignificant.

In terms of other chronic diseases, maternal thyroid disease had a statistically significant influence on the occurrence of ASD in the offspring (20/120 (16.67%) vs. 5/100 (5.0%); $p = 0.009$). Other diseases in parents were not statistically significant. Similarly, the provision of specialist care to parents did not increase the risk for ASD in the offspring.

Other factors (conception problems, history of previous pregnancy and miscarriage, mean time since the previous pregnancy and conception with assisted reproductive techniques) were observed with a comparable frequency in groups 1 and 2 with no statistically significant influence on the risk of ASD.

**Table 2.** Potential preconception risk factors for autism spectrum disorder (ASD) in mothers of children from the study and reference groups.

| Risk Factor | Response | Study Group $n$ = 121 (100%) | Reference Group $n$ = 100 (100%) | Significance Level |
|---|---|---|---|---|
| conception problems | Yes | 16 (13.22%) | 12 (12.0%) | $p = 0.47$ |
| | No | 105 (86.78%) | 88 (88.0%) | |
| assisted reproductive techniques | Yes | 2 (1.65%) | 5 (5.0%) | $p = 0.25$ |
| | No | 119 (98.35%) | 95 (95.0%) | |
| another pregnancy | Yes | 54 (44.63%) | 52 (52.0%) | $p = 0.28$ |
| | No | 67 (55.37%) | 48 (48.0%) | |
| time since the previous pregnancy (in months) | | $n = 46$ | $n = 47$ | $p = 0.53$ |
| | mean | 55.9 | 49.1 | |
| | standard deviation | 49.9 | 42.0 | |
| | median | 36 | 29 | |
| | Min–max | 3–168 | 6–144 | |
| previous miscarriages | Yes | 12 (9.92%) | 11 (11.0%) | $p = 0.48$ |
| | No | 109 (90.08%) | 89 (89.0%) | |
| oral contraception | Yes | 56 (46.28%) | 29 (29.0%) | **$p = 0.01$** |
| | No | 65 (53.72%) | 71 (71.0%) | |
| duration of oral contraception | | $n = 56$ | $n = 29$ | |
| | ≤1 year | 11 (19.64%) | 8 (27.59%) | $p = 0.42$ |
| | >1 year | 45 (80.36%) | 21 (72.41%) | |
| mental illness in the mother/mother's family | absent | 78 (64.46%) | 84 (84.0%) | |
| | ASD * | 8 (6.61%) | 3 (3.0%) | **$p = 0.0002$** |
| | other * | 35 (28.93%) | 13 (13.0%) | |
| chronic conditions | thyroid disease | 20 (16.67%) | 5 (5.0%) | **$p = 0.009$** |
| | cardiovasculardisease | 4 (3.33%) | 1 (1.0%) | $p = 0.38$ |
| | ophthalmic diseases | 3 (2.50%) | 2 (2.0%) | $p = 0.99$ |
| | arterial hypertension | 4 (3.33%) | 0 | $p = 0.13$ |
| | epilepsy | 2 (1.67%) | 0 | $p = 0.50$ |
| | diabetes | 2 (1.67%) | 0 | $p = 0.50$ |
| | other | 39 (32.50%) | 29(29.00%) | $p = 0.18$ |
| care in the specialized outpatient clinic | endocrinology | 17 (14.17%) | 7 (7.0%) | $p = 0.13$ |
| | cardiology | 4 (3.33%) | 3 (3.0%) | $p = 0.99$ |
| | ophthalmology | 4 (3.33%) | 2 (2.0%) | $p = 0.69$ |
| | neurology | 9 (7.50%) | 3 (3.0%) | $p = 0.23$ |
| | diabetology | 0 | 0 | - |
| | other | 18 (15.0%) | 13 (13.0%) | $p = 0.70$ |

* counted together for the statistical analysis; Statistically significant figures are marked in bold.

**Table 3.** Potential preconception risk factors for ASD in fathers of children from the study and reference groups.

| Risk Factors | Response | Study Group $n$ = 120 (100%) | Reference Group $n$ = 100 (100%) | Significance Level |
|---|---|---|---|---|
| mental illness in the father/father's family | absent | 86 (71.67%) | 70 (70.0%) | |
| | ASD * | 5 (4.17%) | 2 (2.0%) | $p = 0.88$ |
| | other * | 29 (24.17%) | 28 (28.0%) | |
| chronic conditions | thyroid disease | 1 (0.83%) | 4 (4.0%) | $p = 0.18$ |
| | cardiovascular disease | 0 | 0 | - |
| | ophthalmic diseases | 10 (8.33%) | 5 (5.0%) | $p = 0.42$ |
| | arterial hypertension | 0 | 3 (3.0%) | $p = 0.10$ |
| | epilepsy | 1 (0.83%) | 2 (2.0%) | $p = 0.59$ |
| | diabetes | 0 | 0 | - |
| | other | 36 (30.00%) | 24 (24.0%) | $p = 0.36$ |
| care in the specialized outpatient clinic | endocrinology | 1 (0.83%) | 4 (4.0%) | $p = 0.18$ |
| | cardiology | 2 (1.67%) | 3 (3.0%) | $p = 0.66$ |
| | ophthalmology | 2 (1.67%) | 2 (2.0%) | $p = 0.99$ |
| | neurology | 3 (2.50%) | 0 | $p = 0.25$ |
| | diabetology | 0 | 0 | - |
| | other | 14 (11.67%) | 13 (13.0%) | $p = 0.64$ |

* counted together for statistical analysis.

**Table 4.** Relative risks for significant factors.

| Factor | Relative Risk (RR) | 95% Confidence Interval (CI) | Significance Level |
|---|---|---|---|
| oral contraception | 1.38 | 1.09; 1,74 | $p = 0.007$ |
| maternal chronic thyroid disease | 1.56 | 1.23; 1.98 | $p = 0.0003$ |
| mental illness in the mother/mother's family | 1.51 | 1.21; 1.89 | $p = 0.0003$ |

## 4. Discussion

The study showed a statistically significant effect of three preconception risk factors for ASD in offspring, i.e., mental illness in the mother/mother's family, maternal thyroid disease and the use of oral contraception.

There are reports on the correlation between ASD and parental psychiatric disorders. A family history of psychiatric illness was associated with higher odds of ASD in the index persons. An occurrence of ASD, intellectual disability, attention deficit/hyperactivity disorder, obsessive compulsive disorder, schizophrenia and other non-affective psychotic disorders, depression, bipolar disorder and personality disorder was found. The more closely related the affected family member was, the higher the odds were of ASD for the index person. At the same time, ASD without mental retardation was evidently associated with more disorders compare with ASD with an intellectual disability [19]. The association between maternal mental illness and ASD observed in the present study is consistent with this study from the literature.

In an Australian study, compared with mothers with no previous psychiatric contact, those with any psychiatric contact were 2.5-times as likely to have a child with ASD without an intellectual disability and more than twice as likely to have a child with ASD with an associated intellectual disability [20]. Swedish population studies showed a 2-fold higher prevalence of ASD among children of mothers with a psychiatric illness and fathers treated for schizophrenia. Parent diagnoses were based on an inpatient hospital diagnostic evaluation and included schizophrenia, other non-affective psychoses, affective disorders, neurotic and personality disorders and other nonpsychotic disorders, alcohol and drug addiction and abuse, and autism [21]. Similarly, Lauritsen et al. in their study on the Danish population observed that the risk of ASD was twice as high among children whose mothers were diagnosed with a psychiatric disorder compared with children of mothers with no history of psychiatric illness [22]. In addition, the risk of ASD associated with maternal antidepressant exposure during the pre-pregnancy period vs. all unexposed women appeared statistically significantly elevated and was similar in size to that of exposure during pregnancy [23].

The authors did not find data in the literature on the relationship between the occurrence of maternal thyroid disease in the preconception period and the development of ASD in children. However, a statistically significantly increased risk for ASD was observed in the offspring in mothers with hypothyroidism in pregnancy. It was found that the odds of being a probable autistic child at the age of 6-years-old increased almost 4-fold when the mother had severe hypothyroidinemia (defined as $0.03 < \text{TSH} < 2.5$ mIU/L and $fT_4 < 10.99$ pmol/L) in early gestation [24]. Maternal hypothyroidism diagnosed and treated for the first time after the birth of the child increased the risk of ASD, whereas no significant association was seen for a maternal diagnosis and treatment prior to the birth of the child [25]. As a risk factor for ASD, autoimmune thyroiditis was also reported in pregnant women. The prevalence of maternal anti-thyroid peroxidase antibodies was significantly increased in pregnancies giving rise to autism cases compared with controls. The odds of autism were increased by nearly 80% among the offspring of mothers who had positive anti-thyroid peroxidase antibodies during pregnancy, compared with mothers negative for this autoantibody. The measures of maternal thyroid hormones did not differ between these groups [26]. Therefore, thyroid disorders in women who plan pregnancy should be effectively treated. More research is warranted to assess the impact of the disorders of thyroid metabolism in the preconception period.

In our study, a history of epilepsy, arterial hypertension and other cardiovascular diseases, diabetes, ophthalmic diseases and other chronic diseases did not have a statistically significant effect on the manifestation of ASD. There are no data in the literature on the impact of these diseases during the preconception period. However, studies have demonstrated an increased risk of ASD in children of mothers with hypertension and/or preeclampsia during pregnancy. The adjusted pooled results of the systematic review and meta-analysis indicated that exposure to maternal gestational hypertension was associated with 35% increased odds of ASD compared with nonexposure [27–29]. Similarly, in the case of diabetes, studies have confirmed a statistically significant influence of maternal diabetes on the development of ASD in the offspring, however, without distinguishing preconception glycemic disorders [30–32]. Other studies reported that an increased serum glucose level in a pregnant mother was not a risk factor for ASD [29]. Panjwani et al. reported low maternal high-density lipoprotein cholesterol (HDL-C) and above-median maternal plasma branched-chain amino acid concentrations as risk factors [31].

In our study, parental epilepsy was not a risk factor for ASD. However, in the population-based cohort study of Swedish participants, having a first-degree relative with cerebral palsy or epilepsy was associated with a 2-fold increase in the odds for ASD compared with those with unaffected first-degree relatives. The differences in our results may be explained by the fact that the researchers from this publication found a correlation between neurological diseases and ASD with mental retardation, while the group we studied was entirely in the intellectual norm [19].

There are interesting findings related to contraception and its duration as risk factors for ASD. On an animal model, an exposure to progesterone during pregnancy induces ASD-like behavior in the offspring. The researchers used eight kinds of clinically relevant progestins for prenatal exposure in pregnant dams, and the offspring showed autism-like behavior. Therefore, many potential clinical progestin applications (including oral contraceptive pills and preterm birth drugs), may be risk factors for ASD. The mechanism was an estrogen receptor beta (ERβ) suppression in the amygdale [33]. In a previous study, postmortem middle frontal gyrus tissues (13 ASD and 13 control subjects) were examined with the protein levels measurement and gene expression analysis. The gene expression analysis identified a 35% decrease in the ERβ mRNA expression in the middle frontal gyrus of ASD subjects. In addition, a 38% reduction in the aromatase (CYP19A1) mRNA expression was observed in ASD subjects. Significant decreases in ERco-activators were also found. These results provided the evidence of the dysregulation of ERβ and co-factors in the brains of subjects with ASD [34]. Similarly, prenatal levonorgestrel exposure also induced autism-like behavior in the same mechanism, which was demonstrated in the animal model [35]. However, the Chinese population-based case–control epidemiology study revealed that the use of progesterone (to prevent miscarriage and as a contraceptive at the time of conception or prenatal consumption of progestin-contaminated seafood during the first trimester of pregnancy) was strongly associated with the prevalence of ASD. Additionally, in vivo experiments in rats were conducted to further confirm the findings. The subsequent offspring of progesterone-fed dams showed autism-like behavior, which further demonstrated that prenatal progestin exposure may induce ASD [36]. Moreover, a statistically significantly increased risk of ASD was found in children of mothers treated with progesterone due to conception problems. Progesterone exposure during the critical period of fetal life elevated the risk of ASD, possibly reflecting an epigenetic modification [37]. On the contrary, Lyan et al. in their population study observed no correlation between the pre-gravid use of oral contraceptives and the risk of ASD in offspring. Additionally, they presented ambiguous results about the duration (in years) of oral contraceptives: in a retrospective study, the risk of ASD was statistically significantly associated with longer use, though in the prospective sub-group, the oral contraceptives' duration association was reversed, with a longer duration among mothers of healthy children [38]. Due to the limitations of the available data, it is difficult to draw clear conclusions. Further research is warranted to assess a potential adverse impact of oral contraception on the development of ASD.

The association between the use of assisted reproductive technology and ASD risk in offspring has been explored in several studies, but the results are still inconclusive. The meta-analysis indicated that the use of assisted reproductive technology may be associated with a higher risk of ASD in offspring. An analysis of the total 11 records (3 cohort studies and 8 case–control studies) revealed that the use of ART is associated with a higher percentage of ASD [39]. However, some studies have not demonstrated an increased risk of developing ASD in children conceived using assisted reproductive techniques. In Spain, 231 children conceived by this technique and 208 children conceived naturally under the age of 3 were assessed. No differences were observed in the occurrence of neurodevelopmental disorders (global developmental delay, ASD or speech delay). Based on the analysis of the potential risk factors associated with assisted reproductive techniques, only a correlation between one type of technique (the transfer of frozen embryos) and speech delay was demonstrated, which had not been previously described [40]. Similarly, the study of the Israeli population did not show the effect of in vitro fertilization on the development of ASD compared with the control group of naturally conceived children [37]. Therefore, further prospective, large and high-quality studies are still required.

There are only a few reports on a possible relationship between miscarriage in a previous pregnancy and the manifestation of ASD. In our group of patients with ASD, no correlation was found between a history of miscarriage and the development of ASD in the subsequent children. However, the results of a German study indicated that miscarriage could be a specific risk factor for attention deficit hyperactivity disorder (ADHD) with ASD in children [41].

The authors observed that parents in the reference group are more highly educated than in the study group. So far, it is hard to explain the reason for such an impact. One hypothesis suggests an influence of a healthier lifestyle, however, the authors did not ask about that. Conclusions from various studies assessing the influence of parental education on the risk of autism in offspring are inconclusive [42]. Lee et al. presented findings similar to our results [43]. On the contrary, some studies showed a positive correlation between higher education and ASD [44–46]. Therefore, there is a need for further studies.

The strength of this work is a homogeneous study group of children with autism spectrum disorders without additional comorbidities, where a group called "pure autism" was obtained. This is the optimal group that will permit the creation of endophenotypes for further research, and at this stage allows the optimal selection of children for the control group (healthy children in the intellectual norm). Both groups include Caucasian children living in a similar environment, which is extremely important due to the participation in the formation of autism spectrum disorders of genetic and environmental factors. From the researchers' perspective, the relatively young age of the respondents is important a sit allows the researchers to plan a prospective study in the future, which is a value in itself for population research.

The advantage is also the fact that survey was conducted personally by experienced practitioners and researchers in autistic centers known to children, in which they trust. Parents were not accompanied by children during the study, so they could freely answer the researcher.

A number of limitations should also be noted. The study group for such a common disease is small, where the study at this stage is a form of pilot study, and the conclusions drawn so far will improve the diagnostic tool which is the survey.

The questionnaire is an author's own questionnaire—it was practically used for the first time. A detailed analysis of the data contained in the questionnaire revealed its disadvantages, including that the questions about psychiatric disorders in parents and in families of autistic children were not sufficiently developed—it would be better to enter closed questions about specific psychiatric diseases entered in the family tree.

Moreover, the data, including sensitive data regarding the family's health status, were based on information provided by parents: no pregnancy record card or information regarding the health of the patient's family members were analyzed. Unfortunately, bias due to the interviewers' subjectivity cannot be ruled out on the responses to some of the questions related to pregnancy and/or family history.

Additionally, most of the children who participated in the study and control groups were unrelated, and while there were also several cases of siblings—due to the small study group—this aspect was not analyzed.

The authors in the presented work use only part of the questionnaire. There are still other questions to be analyzed, including correlations in the clinical picture of ASD or comorbidities in ASD in relation to prenatal factors. Further analysis will perhaps allow to determine endophenotypes and perform more detailed research.

**Author Contributions:** Conceptualization, K.B. and E.-W.E.; methodology, K.-G.A.; software, H.M., P.J., K.B.; validation, E.-W.E.; formal analysis, E.-W.E., P.J.; investigation, M.S.-F., B.A.; resources, M.S.-F., A.B.; data curation, H.M.; writing—original draft preparation, E.-W.E., H.M., P.J., K.B.; writing—review and editing, H.M., P.J., E.-W.E. visualization & supervision, E.-W.E., P.J., K.B.; project administration, H.M., K.B., E.-W.E., P.J.; funding acquisition, E.-W.E., P.J. All authors have read and agreed to the published version of the manuscript.

**Funding:** The study was conducted as part of the statutory work of the Medical University of Silesia NPC-1-195/K/9/K.

**Acknowledgments:** The authors wish to thank all patients and their parents for participating in this study.

**Conflicts of Interest:** The authors declare no conflict of interest.

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# Self-Reported Autistic Traits Using the AQ: A Comparison between Individuals with ASD, Psychosis, and Non-Clinical Controls

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Received: 6 April 2020; Accepted: 12 May 2020; Published: 14 May 2020

**Abstract:** The term "autism" was originally coined by Eugen Bleuler to describe one of the core symptoms of schizophrenia. Even if autism spectrum disorder (ASD) and schizophrenia spectrum disorders (SSD) are now considered two distinct conditions, they share some clinical features. The present study aimed to investigate self-reported autistic traits in individuals with ASD, SSD, and non-clinical controls (NCC), using the Autism-Spectrum Quotient (AQ), a 50-item questionnaire. The study was conducted in the Psychiatry Unit of Policlinico "G. Rodolico", Catania, Italy. The AQ was administered to 35 adults with ASD, 64 with SSD, and 198 NCC. Overall, our data showed that the ASD sample scored significantly higher than NCC. However, no significant differences were detected between individuals with ASD and SSD. Notably, the three groups scored similarly in the subscale "attention to detail". AQ showed good accuracy in differentiating ASD from NCC (AUC = 0.84), while discriminant ability was poor in the clinical sample (AUC = 0.63). Finally, AQ did not correlate with clinician-rated ADOS-2 scores in the ASD sample. Our study confirms that symptoms are partially overlapping in adults with ASD and psychosis. Moreover, they raise concerns regarding the usefulness of AQ as a screening tool in clinical populations.

**Keywords:** autism spectrum disorder; psychosis; schizophrenia; psychopathology; AQ; screening; accuracy; attention to detail; self-awareness; insight

## 1. Introduction

The term "autism" was firstly introduced by Eugen Bleuler (1911) to describe one of the core features of schizophrenia. Bleuler described autism as a "loss of contact with reality together with the relative and absolute predominance of the inner life" [1]. During the last century, several connotations were given to the term, until Leo Kanner (1943) described the neurodevelopmental disorder that is now called "autism" [2]. Only during the 1970s, autism and schizophrenia were regarded as very distinct entities [3,4]. Nowadays, autism spectrum disorder (ASD) is diagnosed in the presence of a persistent impairment in communication and reciprocal social interaction as well as restricted, repetitive patterns of behavior, interests, or activities. These symptoms usually occur during early childhood and cause significant impairments in everyday functioning. However, a diagnosis of ASD may be received later in life, when "social demands exceed the limited capacities of individuals" [5,6]. Prevalence estimates of ASD would range around 1.5% of the general population [7,8].

Schizophrenia spectrum disorders (SSD), also referred to as psychotic disorders, include instead a broad range of conditions which onset usually occurs during adolescence or young adulthood. They comprise not only schizophrenia, but also delusional disorder, brief psychotic disorder, schizophreniform disorder, schizoaffective disorder, psychoses induced by drugs or medical conditions, and psychoses not-otherwise-specified. SSD is characterized by heterogeneous symptoms which may vary in intensity and duration, such as hallucinations, delusions, disorganized speech, bizarre behaviors, and social withdrawal [5]. It has been estimated that approximately 1 in 150 individuals is diagnosed with a psychotic disorder at some point during their lifetime [9].

Even if SSD and ASD are currently considered distinct entities, they both represent chronic, multi-factorial disorders. They share genetic predispositions [10,11] and environmental risk factors, such as complications during pregnancy or at birth [12,13]. Moreover, they present with similar neuroimaging patterns [14,15], neurochemical abnormalities, such as dopaminergic dysregulations [16,17], and inflammatory pathways [18].

Both ASD and SSD show disturbances in several psychopathological domains; these alterations are similar in some cases, opposite in others [19]. First, content-thought disturbances may present with delusions in psychotic people, while scarce cognitive flexibility is typical of individuals with ASD. Paranoia is common to both conditions. However, in ASD it appears as a direct consequence of social interaction difficulties, rather than a cause of them. In fact, it has been hypothesized that the rigid thinking style may lead to difficulties in social interaction and thus to experience adverse social relations. Such events may produce negative beliefs which in turn lead to the onset of paranoid ideas [20]. Formal thought disturbances, such as the use of atypical or nonsensical language, characterized by tangentiality, circumstantiality, neologisms, are common to both groups [21].

Difficulties in social interaction are pervasive in both conditions and social cognitive deficits could partially explain the difficulties encountered by individuals with ASD and SSD [22]. However, other factors, such as the lack of interest in activities, the flat emotional affect, as well as the presence of thought disturbances (e.g., delusions), may play a critical role in psychoses [23]. A phenomenological analysis of the world–self boundary could help in an accurate differentiation: in fact, people with psychosis have a weak or variable boundary between the self and the world [24], while this limit seems better defined in individuals with ASD [25].

Perceptual alterations manifest in very different ways. Visual or auditory hallucinations are common in patients with psychoses [5], while hypo- or hyper-sensitivity is typical of individuals with ASD (e.g., the attraction for light sources, refusal of foods because of their color, elevated pain tolerance, altered olfactory threshold, etc.) [26,27]. Not by chance sensory alterations have been introduced among ASD core features in DSM-5 [5]. Behaviors might be disorganized in people with psychoses, while individuals with ASD typically feel comfortable with routines and sameness [28]. Nevertheless, mannerisms and stereotypies can occur in schizophrenia as well as in ASD [29]. Again, it is important to underline that the etiology is different. In SSD, mannerisms can emerge from delusional ideas, but may also be regarded as an expression of catatonic motor disorder or a manifestation of negativism [29]. The role of repetitive behaviors and mannerisms in individuals with ASD remains still unclear, although a wide variety of functions have been attributed to them: for instance, they can be used to calm anxiety, to communicate emotions, or for self-stimulatory purposes [30]. Interestingly, in the DSM-5 the specifier "with catatonia" has been introduced for ASD [5].

Even if ASD and SSD are currently considered two clearly distinct disorders, misdiagnoses are not infrequent, as clinicians who are not familiar with ASD may be misled by some peculiar features. For instance, the lack of meaningful relationships might be interpreted as an expression of negative symptoms (SSD) rather than a real difficulty in social interaction (ASD). Analogously, paranoia could be misjudged as an actual delusion (SSD) rather than a consequence of the difficulties in social cognition and theory of mind (ASD). Interestingly, Geurts et al. [31] reported that 9% of adults who received an ASD diagnosis in adulthood had been previously diagnosed with psychosis; this proportion was much higher (26%) in a study conducted by Nylander and Gillberg [32]. The co-occurrence of the

two conditions represents another critical issue, as autistic symptomatology may be partially covered by a comorbid psychosis. In fact, on one hand, recent meta-analyses have reported that the pooled prevalence of SSD in individuals with ASD would range around 4% [33], 6% [34] or 9.5% [35]. One the other hand, the prevalence rates for autistic-like traits would range from 9.6% to 61% in psychotic patients, whilst the prevalence rates for diagnosed ASD ranged from <1% to 52% [36].

The numerous overlapping features between ASD and SSD may explain why people in the psychotic spectrum may misleadingly score above the cut-off in standardized diagnostic tools for ASD, such as the Autism Diagnostic Observation Schedule (ADOS-2) [37], as reported by several studies [6,38,39]. However, formal clinical evaluation for ASD with standardized tools is a long and time-consuming process [40]. Therefore, clinicians and researchers have tried to examine whether self-report instruments, such as the Autism-Spectrum Quotient (AQ) [41], could be useful in screening subjects with suspected ASD to address them to a more exhaustive evaluation. The AQ is a 50-item self-report tool that has been originally developed to measure the degree of autistic traits in adults with normal intelligence, with higher scores indicating more severe symptoms [41]. The AQ can be used for measuring autistic traits in the general population and for clinical screening of individuals with suspected ASD, with different cut-offs [42]. The guidelines of the UK National Institute for Health and Care Excellence (NICE) [40] suggest the use of the AQ-10—a brief version of the AQ [43]—as a screening tool for adults with possible autism. Moreover, the Adult Asperger Assessment (AAA), including the AQ, is suggested as a formal assessment tool to support the diagnosis of ASD in adults with intelligence within the normal range. Indeed, the AQ is used as a screening tool in clinical settings [44,45], as well as for the inclusion of participants in observational and interventional studies [46,47]. Interestingly, a large naturalistic study conducted by Ashwood et al. [48] has recently shown that self-reported AQ scores did not significantly predict receipt of a diagnosis of ASD in adulthood.

Focusing on the differences in AQ scores between ASD and SSD, a recent meta-analysis has found that people with SSD have indeed significantly higher autistic traits than the general population and lower autistic symptoms than individuals with ASD [49]. However, other authors have reported that, even if AQ may represent a reliable screening tool in the general population, its usefulness in identifying ASD in clinical environments is questionable [50–52]. Importantly, to our knowledge, only four papers specifically compared autistic traits in ASD and SSD and evaluated the discriminant ability of AQ between the two conditions, with contrasting findings [53–56]. In light of the inconsistent results regarding the usefulness and accuracy of AQ as screening tool among the general population as well as in psychiatric environments, the present study aimed to:

1. Investigate the differences in self-reported autistic traits between adults with ASD, SSD and a non-clinical control group (NCC) from the general population;
2. Analyze the accuracy of AQ in discriminating between ASD and SSD, as well as between ASD and NCC.
3. Correlate the AQ scores with ADOS-2 scores in the ASD population.

## 2. Materials and Methods

### *2.1. Setting and Procedures*

The present study was conducted in the outpatient service of the Psychiatry Unit of Policlinico "G. Rodolico", Catania, Italy. From January to December 2019, we consecutively recruited 297 participants. Subjects were asked to complete a form containing personal information and to fill out the AQ. Each participant provided written informed consent before any study procedures commenced. The study was performed according to the Declaration of Helsinki and approved by our internal review board before recruitment.

### 2.2. Participants

The total sample comprised of 297 participants. For inclusion in the present study, all participants had to fulfill the following criteria: (1) age ≥ 18 years; (2) absence of intellectual disability or major cognitive impairment; (3) good knowledge of written and spoken Italian language; (4) written informed consent.

Thirty-five subjects had a diagnosis of ASD as confirmed by an exhaustive clinical examination and administration of standardized clinical interviews (i.e., Autism Diagnostic Observation Schedule-2 (ADOS-2) and/or Autism Diagnostic Interview-Revised (ADI-R); see a previous work by Fusar-Poli et al. [6] for detailed procedures). The ADOS-2 is a semi-structured observation of individuals who may belong to the autism spectrum. It is composed of different domains: communication, reciprocal social interaction, communication + social interaction, imagination/creativity, and stereotyped behaviors and restricted interests. The ADOS-2 consists of five modules addressed to children and adults according to their developmental and language levels. All participants included in the present study have been administered Module 4, which has been developed for adolescents and adults with good verbal fluency. For score calculation, we used the original algorithm proposed by Lord et al. [37]. According to the original algorithm, the domain "communication + social interaction" should be used to collocate an individual into the autism spectrum or autism. Of note, the presence of current or past psychiatric comorbid disorders was considered a reason for exclusion from the analysis.

Sixty-four subjects had received a diagnosis of SSD, as confirmed by a clinical evaluation made by at least two medical doctors (one senior psychiatrist and a trainee), and the administration of the Structured Clinical Interview for DSM-5 (SCID-5) [57]. Participants received the following diagnoses: unspecified psychosis ($n$ = 22), paranoid schizophrenia ($n$ = 9), schizoaffective disorder ($n$ = 9), substance-induced psychosis ($n$ = 9), delusional disorder ($n$ = 5), unspecified schizophrenia ($n$ = 5), undifferentiated schizophrenia ($n$ = 2), catatonic schizophrenia ($n$ = 1), residual schizophrenia ($n$ = 1), disorganized schizophrenia ($n$ = 1). None of the subjects were in a florid psychotic state at the moment of study completion, i.e., they did not present severe positive symptoms, profound negative symptoms, significantly disorganized or catatonic behaviors. The presence of ASD was excluded by a clinician with significant expertise in the field after the consultation of patients' history through clinical charts and the direct observation of the subjects.

Finally, we recruited 198 non-clinical controls (NCC) among students and faculty staff members. Participants from the general population were interviewed by a senior psychiatrist using the SCID-5 [57]. People who fulfill the criteria for any psychiatric diagnosis were excluded from the analysis. The socio-demographic characteristics of the participants are reported in Table 1.

**Table 1.** Characteristics of participants.

| | **ASD Group ($n$ = 35)** | **SSD Group ($n$ = 64)** | **NCC ($n$ = 198)** | ***p*-Value** |
|---|---|---|---|---|
| Sex, male (%) | 22 (62.9) | 39 (60.9) | 96 (48.5) | 0.1 |
| Age, mean ± SD (range) | 26.15 ± 6.55 (18–45) | 39.10 ± 14.48 (18–77) | 34.01 ± 11.99 (19–67) | <0.001 * |
| Educational level, $n$ (%) | | | | <0.001 * |
| Primary school | 0 (0) | 8 (12.5) | 0 (0) | |
| Secondary school | 12 (34.3) | 27 (42.2) | 6 (3) | |
| High school | 18 (5.4) | 23 (35.9) | 24 (12.1) | |
| University | 5 (14.3) | 6 (9.4) | 168 (84.8) | |

**Table 1.** *Cont.*

| | ASD Group ($n$ = 35) | SSD Group ($n$ = 64) | NCC ($n$ = 198) | $p$-Value |
|---|---|---|---|---|
| Occupational status, $n$ (%) | | | | <0.001 * |
| Full-time | 5 (14.3) | 9 (14.1) | 114 (57.6) | |
| Part-time | 4 (11.4) | 0 (0) | 14 (7.1) | |
| Unemployed | 14 (40) | 41 (64.1) | 11 (5.6) | |
| Student | 12 (34.3) | 8 (12.5) | 54 (27.3) | |
| Retired | 0 (0) | 6 (9.4) | 5 (2.5) | |
| Marital status, $n$ (%) | | | | 0.004 * |
| Single | 33 (94.3) | 43 (67.2) | 129 (65.2) | |
| In a domestic partnership | 1 (2.9) | 2 (3.1) | 22 (11.1) | |
| Married | 1 (2.9) | 11 (17.2) | 38 (19.2) | |
| Divorced | 0 (0) | 7 (10.9) | 6 (3) | |
| Widowed | 0 (0) | 1 (1.6) | 3 (1.5) | |
| ADOS-2, mean ± SD | | | | |
| Communication | 3.62 ± 1.59 (0–6) | - | - | - |
| Social Interaction | 6.74 ± 2.94 (2–16) | - | - | - |
| Communication + Social Interaction | 10.4 ± 4.23 (2–22) | - | - | - |
| Imagination/Creativity | 0.86 ± 0.65 (0–2) | - | - | - |
| Restricted Interests and Repetitive Behaviors | 1.80 ± 1.30 (0–5) | - | - | - |

ADOS-2 = Autism Diagnostic Observation Schedule-2; ASD = Autism Spectrum disorder; NCC = Non-clinical controls; SSD = Schizophrenia spectrum disorders. * Statistically significant.

### 2.3. Autism-Spectrum Quotient (AQ)

All participants completed the AQ, the adult version, a widely used measure for the identification of autistic traits in the general population. Literature has shown that the reliability and consistency of the AQ are good [42]. The AQ consists of 50 items, rated using a 4-point Likert scale (1 = "definitely agree", 2 = "slightly agree", 3 = "slightly disagree", and 4 = "definitely disagree"). It is composed of five subscales: social skills (SS), communication (C), imagination (I), attention to detail (AD), and attention switching (AS). We used the binary scoring method (the presence of autistic traits, either mildly or strongly, is scored as a +1, while the opposite is scored 0). Using the binary score method, the total score ranges can between 0 and 50, while the score of each subscale can range between 0 and 10. Higher AQ total score indicates higher autistic traits; higher scores in each subscale reflect poor social skills, poor communication skills, poor imagination, strong attention to details, and poor attention switching, respectively.

### 2.4. Statistical Analysis

Data were tested for normal distribution before applying statistical procedures. Continuous variables were reported as means and standard deviations, while dichotomous variables as percentages or counts, as appropriate. Chi-squared tests and one-way ANOVA were used to detect differences in socio-demographic characteristics between participants in the ASD, SSD, and NCC groups. One-way ANOVA was used also to investigate differences in AQ scores between the three groups. For post hoc between-group comparisons, the Tukey HSD test was applied.

Receiver operating characteristic (ROC) analyses were used to evaluate the accuracy of AQ in discriminating ASD from SSD and from NCC. We used the classification proposed by Hosmer et al. [58] for the interpretation of AUC values (0.5 = no discrimination; 0.51–0.69 = poor; 0.7–0.79: acceptable; 0.8–0.89: excellent; ≥0.9 = outstanding). Cohen's k was used to calculate the agreement between clinical

diagnosis and classification with ASDASQ. For data interpretation, we used the cutoffs proposed by Landis and Koch [59] (0 = no agreement; 0–0.2 = slight; 0.21–0.40 = fair; 0.41–0.60 = moderate; 0.61–0.80 = substantial; 0.81–1 = almost perfect agreement).

Results were considered statistically significant at the $p \leq 0.05$ level, and all tests were two-tailed. Statistical analysis was performed using SPSS v. 23.0 software packages (IBM, Armonk, NY, USA).

## 3. Results

### *3.1. Characteristics of the Sample*

We recruited a total of 297 subjects, of which 35 had a diagnosis of ASD, 64 had an SSD, and 198 did not meet the criteria for any psychiatric disorder. The sample was mainly composed of males ($n$ = 157), who represented 52.8% of the sample, with no differences between the three groups. Participants were meanly 34.18 ± 18.57 years old (range 18–77), with the ASD group being younger than the SSD and the NCC groups. Significant differences were found also at the educational level, occupational and marital status. In fact, while ASD and SSD patients had completed mainly secondary or high school, a considerable part of controls had a university degree. Moreover, NCC were mostly employed; conversely, a large proportion of participants with ASD and SSD were unemployed, and 34.3% of individuals with ASD were students. Most participants were single, even if in NCC and SSD groups many subjects were married. The characteristics of participants and the ADOS-2 scores for the ASD group have been reported in Table 1.

### *3.2. Differences in AQ Scores*

Overall, our sample ($n$ = 297) obtained a mean score of 18.60 ± 7.88 at the AQ (range 3–43). The highest scores were obtained in the AS domain (4.65 ± 2.33) and the AD (4.55 ± 2.20) domains. A mean value of 3.32 ± 2.10 was scored in the I subscale, while the SS and C had overall mean scores of 3.09 ± 2.49 and 3.00 ± 2.40, respectively. The distribution of scores in the three groups is depicted in Figure 1.

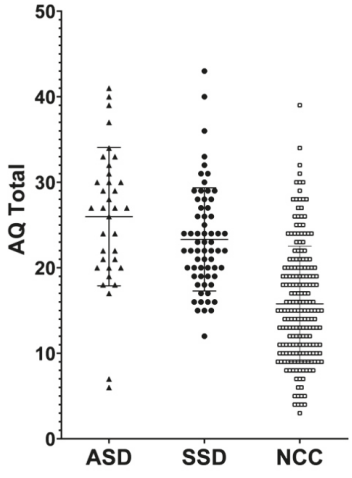


**Figure 1.** Distribution of AutismSpectrum Quotient (AQ) total scores among individuals with autism spectrum disorder (ASD), schizophrenia spectrum disorders (SSD) and non-clinical controls (NCC).

One-way ANOVA detected significant differences between the three groups ($p < 0.001$) except for the AD domain, where no differences were found. However, Tukey HSD post-hoc analysis revealed that while both ASD and SSD significantly differed from NCC in all domains (excluding Imagination),

no significant differences could be found between ASD and SSD patients, neither in the overall AQ score or subscales. The mean and SD for each group and the results of the statistical comparisons have been reported in Table 2.

**Table 2.** AQ scores obtained by each group, and differences between groups.

| | | | | Overall | | ASD vs. SSD | ASD vs. NCC | SSD vs. NCC |
|---|---|---|---|---|---|---|---|---|
| **AQ Scores, Mean ± SD (Range)** | **ASD (*n* = 35)** | **SSD (*n* = 64)** | **NCC (*n* = 198)** | **F** | ***p*** | ***p*** | ***p*** | ***p*** |
| **AQ total** | 25.97 ± 8.09 (6–41) | 23.31 ± 6.03 (12–43) | 15.77 ± 6.75 (3–39) | 53.42 | <0.001 * | 0.15 | <0.001 * | <0.001 * |
| **Social skills** | 5.06 ± 2.55 (0–10) | 4.02 ± 2.41 (0–10) | 2.44 ± 2.23 (0–9) | 25.73 | <0.001 * | 0.08 | <0.001 * | <0.001 * |
| **Attention switching** | 6.31 ± 2.42 (0–10) | 5.66 ± 2.00 (1–10) | 4.03 ± 2.15 (0–9) | 25.59 | <0.001 * | 0.32 | <0.001 * | <0.001 * |
| **Attention to detail** | 4.97 ± 2.17 (1–9) | 4.56 ± 2.22 (1–10) | 4.47 ± 2.21 (0–10) | 0.77 | 0.46 | 0.65 | 0.43 | 0.95 |
| **Communication** | 5.31 ± 2.31 (0–10) | 4.48 ± 2.21 (1–10) | 2.11 ± 1.93 (0–9) | 58.63 | <0.001 * | 0.13 | <0.001 * | <0.001 * |
| **Imagination** | 4.31 ± 1.74 (1–7) | 4.59 ± 1.81 (0–8) | 2.73 ± 2.00 (0–10) | 27.74 | <0.001 * | 0.77 | <0.001 * | <0.001 * |

AQ = Autism-spectrum quotient; ASD = Autism Spectrum disorder; NCC = Non-clinical controls; SSD = Schizophrenia spectrum disorders; * Statistically significant.

### *3.3. Analysis of Accuracy*

ROC curves showed that AQ had an excellent accuracy in differentiating individuals with ASD from NCC (AUC = 0.84, CI 95% 0.76–0.92, $p < 0.001$). On the contrary, the accuracy of AQ in discriminating individuals with ASD from SSD was poor (AUC = 0.63, 95% CI 0.51–0.75, $p = 0.03$). ROC curves are reported in Figure 2.

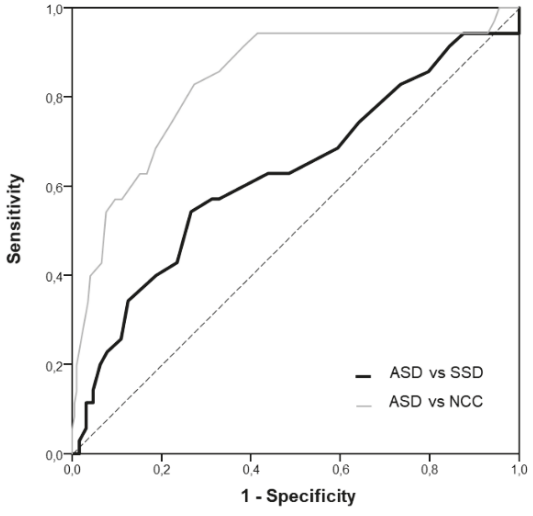


**Figure 2.** Receiver operating characteristic (ROC) curves of AQ total score.

Table 3. reports the values of AUC, sensitivity, specificity, positive (PPV) and negative predictive values (NPV), and agreement with the diagnostic category. Notably, the agreement with the clinical group was fair in the case of NCC (k = 0.45) and null in the case of SSD (k = 0.04). For calculation,

we considered a cut-off of ≥26 for the NCC group and ≥32 for the SSD group, as proposed by Ruzich et al. [42].

**Table 3.** Accuracy of AQ in discriminating ASD from SSD and NCC.

| | AUC (95% CI) | Sensitivity | Specificity | PPV | NPV | Cohen's k |
|---|---|---|---|---|---|---|
| **ASD vs. SSD** | 0.63 (0.51–0.75) | 22.9% | 92.2% | 61.5% | 68.6% | 0.04 |
| **ASD vs. NCC** | 0.84 (0.76–0.92) | 57.1% | 90.4% | 51.3% | 92.3% | 0.45 |

ASD = Autism spectrum disorder; AUC = Area Under Curve; CI = Confidence Interval; NCC = Non-clinical controls; NPV = Negative predictive value; PPV = Positive predictive value; SSD = Schizophrenia spectrum disorders.

### *3.4. Correlation between AQ and ADOS-2 Scores*

We computed Pearson's correlation coefficients (r) to evaluate the correlation between AQ and ADOS-2. Substantially, we did not find any significant correlation, except for those between the AQ Imagination subscale and the social interaction, communication + social interaction and imagination domains of ADOS-2. The correlation matrix has been reported in Table 4.

**Table 4.** Correlations between AQ and ADOS-2 scores.

| | | ADOS-2 | | | | |
|---|---|---|---|---|---|---|
| | | **Communication** | **Social Interaction** | **Communication + Social Interaction** | **Imagination/ Creativity** | **Repetitive Behaviors** |
| **AQ** | **Total** | −0.09 $p = 0.59$ | −0.01 $p = 0.93$ | −0.03 $p = 0.84$ | −0.09 $p = 0.59$ | −0.02 $p = 0.89$ |
| | **Social skills** | −0.32 $p = 0.06$ | −0.24 $p = 0.16$ | −0.29 $p = 0.09$ | −0.28 $p = 0.11$ | −0.22 $p = 0.21$ |
| | **Attention switching** | −0.03 $p = 0.87$ | −0.01 $p = 0.99$ | −0.01 $p = 0.96$ | −0.09 $p = 0.59$ | 0.1 $p = 0.57$ |
| | **Attention to detail** | 0.001 $p = 0.99$ | −0.05 $p = 0.76$ | −0.01 $p = 0.98$ | −0.11 $p = 0.54$ | 0.03 $p = 0.87$ |
| | **Communication** | 0.11 $p = 0.53$ | 0.03 $p = 0.87$ | −0.02 $p = 0.91$ | −0.09 $p = 0.59$ | −0.15 $p = 0.40$ |
| | **Imagination** | 0.21 $p = 0.21$ | 0.37 $p = 0.03$ * | 0.35 $p = 0.04$ * | 0.43 $p = 0.01$ * | 0.29 $p = 0.09$ |

* Statistically significant correlations with $p < 0.05$.

## 4. Discussion

Our study examined the differences in AQ scores between individuals in the autism spectrum, in the schizophrenia spectrum and individuals from the general population, as well as the accuracy of the AQ in discriminating between the different groups. Our data showed that while AQ may represent a good instrument to detect autistic features among the general population (AUC = 0.84), it is not able to correctly discriminate between ASD and SSD (AUC = 0.63), with no significant differences either in the total score or in single subscales. Our results are in contrast with a recent meta-analysis [49] which found that patients in the SSD had lower autistic traits than ASD, but similar to the findings of Lugnegård et al. [55], who reported no significant differences in self-reported AQ scores between autistic and psychotic patients while using the full AQ scales, and poor discriminant validity of the questionnaire (AUC = 0.65).

The more reasonable explanation of our results is that ASD and SSD features are partially overlapping. In fact, the AQ evaluates areas which are typically impaired in both conditions, such as

deficits in socio-communication, attention, and imagination. As mentioned above, abnormalities in verbal and non-verbal communication as well as in social cognition are common to both ASD and SSD. Attention switching, that is the capacity of an individual to flexibly shift mental set to different cognitive demands, is impaired in people with ASD, probably because the restriction of interests hampers them to switch between multiple clues [60,61]. Individuals affected by SSD show analogous impairment in switching attention, even if researchers have not yet clarified whether they should be ascribed to a primary deficit of attention or should be considered secondary to the emergence of delusions, or the experience of hallucinations [62]. Imagination represents instead "the faculty or action of forming new ideas, or images or concepts of external objects not present to the senses, typically derived from creative integration of past experiences, learning, or other information" [63]. Imagination is thought to be limited in individuals with autism, while over-developed in schizophrenia [64]. One can think about the "fantasy life" which characterized Bleuler's autism [1]. However, as suggested by Spek and Wouters [65], most items of the AQ imagination subscale refer to active and purposeful imagination, i.e., "I find it difficult to imagine what it would be like to be someone else". Despite the over-developed imagination in schizophrenia, active control in this respect has been found limited [66], and this could explain while this scale is not able to differentiate ASD from SSD.

Interestingly, in the ASD sample, the AQ scores of the scale regarding "attention to detail" (AD) did not significantly differ from SSD neither from the non-clinical group. Our finding is conflicting with the previous work by Lugnegård et al. [55], which instead found that ASD scored significantly higher in the AD domain than SSD and NCC. While they hypothesized that this subscale may comprise more ASD-specific items, we could not confirm this assumption, as our ASD sample scored similarly to the other groups. One potential explanation is that Lugnegård et al. have recruited subjects with DSM-IV Asperger's syndrome, while our sample was composed of people with a DSM-5 diagnosis of ASD, thus including individuals with higher symptoms severity, even in presence of an IQ in the average range (the presence of ID was an exclusion criterion). Another explanation could be related to the different sex distribution, since in Lugnegård et al. the ASD sample comprised mainly women (51.9%), while our sample was predominantly composed of men (62.9%). However, this is just a speculation, and it is worth underlying that other authors did not find significant differences between ASD and SSD in the AD domain [65].

Another potential reason for our global findings is that the use of a self-report questionnaire, such as the AQ, may not be reliable in clinical contexts. It has in fact been reported that psychiatric patients—above all people in the schizophrenia spectrum—frequently present low levels of insight and tend to under- or over-report their symptoms [67,68]. Lack of self-awareness has been reported also in the population with autism, especially in the presence of greater functional impairment [69]. In fact, the use of self-report measures in the ASD population—including the AQ—has been questioned [70,71]. This hypothesis is partially confirmed by the low sensitivity shown by the AQ, which means a high rate of false negatives. In fact, according to our data, sensitivity was 22.9%, meaning that 77.1% of the ASD sample did not score above the cut-off suggested for clinical samples (≥32). Sensitivity improved (57.1%) while examining the accuracy of AQ un the general population, using the proposed cut-off of ≥26. This result sheds light on a significant limitation of the AQ, since a high sensitivity is clearly important for a screening tool. Nevertheless, it is worth underlying that the AQ has been developed as a descriptive, rather than a diagnostic measure of autistic traits, and for screening purposes rather than for differential diagnosis [41,42].

The poor insight of ASD participants may also explain why the AQ scores in our sample did not correlate with ADOS-2 scores. The ADOS-2 consists of a semi-structured observation of the individual's behaviors and is rated by trained clinicians, not a self-reported tool. This finding is consistent with previous studies [48,52,72] which found no significant correlations between AQ total and ADOS-2 Module 4 scores. Conversely, it has been reported that AQ scores show reliable correlations with measures of anxiety, depression and alexithymia, suggesting that this instrument

may be sensitive to non-specific mental-health vulnerabilities rather than to the defining characteristics of ASD specifically [73].

Despite the importance of our findings, several limitations should be highlighted. First, the sample size, especially the ASD group, was quite small; nevertheless, we have planned to enlarge our sample in future studies to replicate or disconfirm our findings. Second, the ASD group was younger than the SSD and NCC groups as we could match for sex, but not for age. Moreover, given the limited number of participants, we could not perform separate analyses based on sex. Some authors have argued the existence of a "female autistic phenotype", according to which females in the autism spectrum may present with peculiar features, different from their male peers [74]. It would be interesting in future research to evaluate if screening tools, such as the AQ, work better with men or women. Third, we did not conduct a naturalistic study evaluating the predictive value of AQ for a subsequent diagnosis of ASD, as in Aswhood et al., for instance [48]. AQ questionnaires were administered only to individuals with ASD or psychoses, while no other psychiatric disorders were considered. For instance, obsessive-compulsive disorder or personality disorders present overlapping features with ASD, and the examination of AQ accuracy in these groups of patients would be equally useful. Finally, our study was conducted in a single Psychiatry Unit in Italy, therefore we cannot assure cross-cultural generalizability of our results.

## 5. Conclusions

Our study confirmed that the AQ may be useful in discriminating individuals with ASD from non-clinical controls. Nevertheless, it should be cautiously used for ASD screening in clinical populations, especially in the presence of psychotic patients. As suggested by other authors, AQ alone should not be used to exclude further ASD assessment other than if the scores are extremely low [52]. Therefore, the adoption of the AQ as a clinical tool (as recommended by NICE Guidelines [40]) may need to be reconsidered and adapted to different populations [48]. Future studies should investigate the intriguing relationship between insight and self-reported autistic traits. Furthermore, it would be interesting to evaluate the relationship between self-reported and clinician-rated measures in adults with ASD.

**Author Contributions:** L.F.-P. conceived the study, performed data analysis, and wrote the first draft of the manuscript. A.C. contributed to writing the first draft of the manuscript. A.G., V.M., F.P., A.R., G.S., and L.V. participated in data collection and critically contributed to the interpretation of the statistical analysis. A.P., M.S.S. and E.A. participated in data collection, supervised the project and contributed to the manuscript draft. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

**Conflicts of Interest:** All authors declare that they have no conflicts of interest.

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# Changes in Developmental Trajectories of Preschool Children with Autism Spectrum Disorder during Parental Based Intensive Intervention

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Received: 11 April 2020; Accepted: 11 May 2020; Published: 12 May 2020

**Abstract:** Background: Research highlights the positive effects of early intensive intervention with parent and school involvement for preschool children with Autism Spectrum Disorder (ASD) on general developmental outcomes and social skills in randomized controlled trials. However, given the inter-individual variability in the response to treatment, it is necessary to investigate intervention effects in terms of mediators and moderators in order to explain variability and to highlight mechanisms of change. Methods: 25 children in the experimental group were exposed to early intensive intervention and 14 children in the control group were subjected to "as usual" intervention. The initial assessment was obtained at the time of diagnosis (T1) and the follow-up assessment was conducted after 15 months of intervention (T2) in both groups. Results: Participants in the experimental group achieved more prominent gains in both cognitive and socio-interactive skills. The role of specific factors able to predict general quotient and language quotient after intervention were investigated, pointing out the contribution of personal–social and performance abilities. Conclusions: The findings support the importance of parental involvement in targeting ASD core symptoms. Further, results informed our understanding of early predictors in order to identify specific elements to be targeted in the individualized intervention design.

**Keywords:** Autism Spectrum Disorder (ASD); early intensive intervention; developmental trajectories; moderators and mediators of intervention.

## 1. Introduction

Autism Spectrum Disorder (ASD) is defined as a set of neurodevelopmental disorders (DSM-5) that impact on children's development by disrupting socioemotional reciprocity and producing a set of restricted repetitive patterns of behaviours and interests [1]. According to the Centres for Disease Control, about 1 of 59 children were diagnosed with ASD [2]. Psychoeducational intervention for children with Autism Spectrum Disorder (ASD) currently represents a main strategy to achieve symptoms reduction, promoting better adaptation and developmental outcomes [3]. Therefore, the increased prevalence of ASD led to a growing attention to early intervention research.

Different models of intervention started to prove their efficacy in randomised controlled clinical trials, together with longitudinally stable and generalizable outcomes [4–8]. Further, in line with this, a recent study review underlines how developmental interventions improve some specific areas, particularly socio-communicative domain in children with ASD [9]. Considering both efficacy and effectiveness of intervention, areas of improvement include IQ scores, verbal and non-verbal communication measures, adaptive behaviour and social and self-skills but there is less evidence of a significant impact on core autistic symptoms [10,11]. In line with this, specific improvement of

core autistic symptoms has rarely been reported, mainly due to the lack of scalable and quantifiable autism-specific treatment response measures, and due to the fact that standardized diagnostic instruments are not sensitive enough to detect changes after intervention [12–15]. While overall group improvements may be evident, the rate and the nature of these improvements is highly variable across individual differences in children with ASD [16]. Studies on efficacy show, in fact, great inter-individual variability in the response. Some children respond well to treatment (high-responders), whereas other children respond less to the same model of intervention (low- or non- responders) [17,18]. Variability in ASD in fact, not only concerns clinical expressions but also intervention outcomes [19]. Hence, it is difficult to identify one kind of intervention with the highest degree of efficacy compared to others, given that a specific intervention can be useful for specific domains and patients but not for others [12,13,20]. Despite this, treatments share some common principles: precocity, intensity, individualisation and integrated work [20–23].

To conclude, a great amount of research reported the efficacy of different kinds of intervention, underlying improvement of specific skills and highlighting the fundamental role of personalisation. For this reason, current research is focused on developmental trajectories of children with ASD during intervention [24–27]. The role of specific factors influencing intervention response need further investigation [28]. Some evidence indicates that factors associated with different responses include pre-treatment cognitive abilities [10,19,29,30], symptoms severity [31], adaptive skills [30,32], younger age [33], communication abilities [34], play skills [35,36], interest in objects [37], joint attention [36] and imitation [31].

Overall, studies on developmental trajectories focused on cognitive and/or adaptive functioning and symptoms severity pointing out different trends. Cognitive and/or adaptive skills showed major improvements compared to symptoms severity that are demonstrated to be more persistent [19,38,39]. Further, there is consensus regarding the importance, as prognostic factors, of IQ and speech level measured at the beginning of intervention. The level of language development is an important variable that has long been considered a predictive factor of child's outcomes [40,41].

In particular, children who received an intervention targeting early social intersubjective abilities have shown greater long-term language improvements than children in a control group [42]. Recent literature on developmental early intensive intervention focused mainly on interactive pleasure and exchange as a fertile ground to acquire competencies. In line with this, intervention intensity into the therapy room is not able to guarantee generalization of competencies if family and school are not encouraged to take an active role. Parents and school educators are, therefore, involved into the intervention program in order to generalize acquired competencies in more naturalistic settings. Further, there is some evidence that only children without intellectual disabilities at baseline were able to transfer the acquired socio-communication skills into daily life, therefore generalizing them [19]. In the Italian context, school represent a social opportunity in order to increase appropriate stimulations

In order to investigate developmental trajectories, we considered the learning rates, calculated as the difference between mental ages before intervention and after intervention and the time elapsed. It represents an alternative tool to measure change in studies of early intervention [43]. Through these indexes, it is possible to compare developmental profiles throughout time, not only at an absolute level but also taking into account the time elapsed between the two assessments with regard to the typical developmental trajectory. It clearly represents changes in age-equivalents over time and it is more appropriate when intervention lengths of time are similar, but not perfectly equal. Further, it represents an advantage when children functioning's are compared at different chronological ages. In fact positive learning rates mean that the child is narrowing the developmental gap. On the contrary, negative learning rates indicate a wider gap in the developmental trajectory. Learning rates may be useful for both outcome studies and progress representation of specific children functions [44], given that the value can be easily compared among them.

For the reasons expressed above, the purposes of the present work were: (1) to compare developmental trajectories for children receiving a parental based intensive intervention that provides

5–6 h per week, with both family and school involvement, with children exposed to "as usual" intervention, that provides 2–3 h per week of rehabilitative activities delivered by community services (see Methods' section for details); (2) to compare developmental trajectories of children with cognitive functioning equal or above 70 points at general quotient with children with cognitive functioning below 70 points at general quotient in both groups (3) to investigate the relationship between child pre-treatment characteristics and developmental trajectories. We had the following hypotheses in relation with the described objectives. First, we expected to find an overall increased level in cognitive abilities in both groups, however, we hypothesized a greater increase considering children exposed to early intensive intervention with family and school involvement, compared to children exposed to "as usual" intervention. Specifically, in relation to the intervention principles we hypothesized an increased level of linguistic skills. Secondly, we tried to identify a decreased level of autistic symptomatology, in particular considering the socio-communicative area, given the stability throughout the development of the restrictive and repetitive behavioural pattern [27,45]. Thirdly, consistently with previous studies [19,39], we expected that children without cognitive impairment showed major improvements in the developmental trajectory, compared to children with cognitive impairment. Finally, we hypothesized that specific child's variables might influence the developmental trajectory, specifically the chronological age and linguistic abilities at the beginning of the intervention were considered.

## 2. Materials and Methods

### 2.1. Participants

This study involved 25 children with Autism Spectrum Disorder (ASD) (M chronological age = 39.76 months, SD = 10.22; M mental age = 27.92 months, SD = 9.19) exposed to early intensive treatment with parent and school involvement delivered by ODFLab and 12 children with ASD (M chronological age = 45.33 months, SD = 8.34; M mental age = 33.17 months, SD = 12.80) subjected to "as usual" treatment delivered by community services in other regions after a diagnostic assessment at ODFLab (Table 1). All participants were recruited at ODFLab, a clinical and research centre of the Department of Psychology and Cognitive Science—(University of Trento) specialised in functional diagnosis of neuro developmental disorders, especially ASD, where families usually turn to in order to assess children's clinical profile. Moreover, the laboratory employed and currently delivers early intensive intervention with a developmental perspective in the local community [46]. Families coming from other regions usually turn to ODFLab only for the first assessment and monitoring of developmental trajectories every year. The intervention is therefore carried out in their local community services. All families involved in this project were adequately informed about procedure and agreed with a written informed consent. They were also aware of the possibility to drop out from the study in every moment.

**Table 1.** Demographic statistics.

| | Intervention Group M (SD) | Control Group M (SD) |
|---|---|---|
| Chronological age (months) | 39.76 (10.22)<br>range (23–46) | 45.33 (8.34)<br>range (34–59) |
| Mental age (months) | 27.92 (9.19)<br>range (14–56) | 33.17 (12.80)<br>range (9–54) |
| SES (index) | 36.36 (13.90) | 46.69 (20.35) |

The diagnosis of ASD was confirmed through clinical judgment by an independent clinician based on the DSM-5 criteria for Autism Spectrum Disorder, as well as through the Autism Diagnostic Observation Schedule (ADOS-2) [47].

The linguistic mental age was assessed through "Language and Communication subscale" of the Griffith Mental Development Scales. Considering the intervention group the average is 22.76 months (SD = 14.16) and for the control group the average is 27.75 months (SD = 13.51).

The socioeconomic status (SES) of the families, calculated with the Four-Factor Index of Social Status [48], indicated a middle status in the intervention group and a middle-high status in the control group.

### *2.2. Procedure*

All procedures of our study were in accordance with the ethical standards of the Italian Association of Psychology (AIP) and with the ethical standards of the Ethics Committee of the University of Trento (Italy) and the last version of Declaration of Helsinki [49]

In order to determine children's developmental level, the Griffith Mental Development Scale-Edition Revised [50] was administered to all children. Children were classified as "children without cognitive impairment" if they received a score equal or above 70 on the general developmental quotient and as "children with cognitive impairment", if they received a score lower than 70. In the experimental group, fourteen children (56%) were classified as children without cognitive impairment and 11 children (44%) were classified as children with cognitive impairment. Considering the control group, six children (50%) were classified as children without cognitive impairment and six children (50%) were classified as children with cognitive impairment. Taking into account the level of language development and the chronological age of children, ADOS Toddler, Module 1 and Module 2 were used to certify the presence of Autism Spectrum Disorder and to specify the severity level.

These measures (see measures' section for details) were applied before intervention (T1), during the first diagnostic and functional assessment. After intervention (T2), children were re-assessed in order to investigate developmental trajectories pre- and post-intervention, considering both cognitive and socio-interactive aspects. For participants in the experimental group (M = 14.72 months, SD = 4.36) and participants in the control group (M = 16.67 months, SD = 4.47) the amount of elapsed time, around fifteen months, is comparable.

### *2.3. Measures*

#### 2.3.1. Griffiths Mental Development Scales-Edition Revised

The Griffiths Mental Development Scale, Edition Revised [50] was used to assess children's mental development level. The GMDS-ER are developmental scales normalized also in an Italian sample and are administered by trained psychologists to the child in a laboratory setting through standardized activities designed to evaluate different aspects of mental development in infants and children, providing scores relative to 6 subscales: Locomotion; Personal–Social; Communication and Listening; Eye–Hand Coordination; Performance; and Practical Reasoning. This scale provides a global quotient and a developmental age-equivalent—allowing to detect developmental delays—as well as specific quotients and developmental age-equivalents for each of the 6 subscales. Both global score and subscale scores were taken into account for the purposes of the present study.

#### 2.3.2. Autism Diagnostic Observation Schedule-2 (ADOS-2)

In the present study, we used the Autism Diagnostic Observation Schedule-2 (ADOS-2) [47] both to confirm participants' diagnosis, to measure symptoms severity, and to investigate patterns of change before and after intervention. The administration of this tool is carried out by trained psychologists after an official ADOS course. For the purposes of this study, we used Toddler Module, Module 1 and Module 2. Each module gives a final score that classifies the child into mild, moderate or severe form of symptoms. Both global score and scores considering social-affect area and restricted, repetitive behaviours area are taken in consideration for the purposes of the present study.

### 2.4. Models of Intervention

#### 2.4.1. Parental Based Intensive Intervention

ODFLab (Observation, Diagnosis and Educational Laboratory) proposes and currently applies an "Italian Model of Intervention" which integrates empirically validated scientific principles together with guidelines in accordance to the Italian sanitary system and organization of educative system that guarantees a specialized educator for classrooms with children with special needs [22,46,51]. The intervention is individualized, comprehensive and integrates behavioural, developmental and relationship-based principles, according to the basic concepts of the Early Start Denver Model [10,13]. This intervention promotes Intentionality by giving to a child behaviour a communicative value so that he/she experiments that an action influences others behaviour and Reciprocity, starting from child behaviour to build up exchanges based on shift alternation. The therapist's goal is, therefore, to facilitate intentionality and reciprocity for children and share them with parents and educators. Further, intervention goals are constantly monitored and changed depending on the child's developmental improvements. Trained therapists aim constantly to create pleasant relationships starting from a child's own pleasure during shared activities [22].

The intervention is focused on the activation of interactive circuits during communication and on acquisition of specific functional competencies through psycho-educative activities. The intervention identifies key target areas and comprises specific activities and related objectives that are progressively adapted based on a specific observational schedule. This is regularly filled in by the psychologists to monitor the learning trajectory and disclose emergent abilities to be targeted during the intervention. Hence, the activities are highly integrated into playful routines to promote the development of specific objectives (e.g., language) by means of a comprehensive work on emergent abilities (e.g., communicative gestures or imitation). These principles are in line with Early Start Denver Model and more generally with Naturalistic developmental behavioral interventions [9,10]. In order to strengthen the generalization of child competencies it is fundamental to involve caregivers into the therapeutic setting from the beginning. In fact, caregivers represent a child's main interactive partners who, if they adequately learn appropriate interactive strategies, may effectively exploit them in more naturalistic settings. To this end, caregivers are involved in a child's social routines as an active part during intervention. For the same reason, they are fundamental to help school educators in understanding and responding to child behavior and structuring adequate activities. Moreover, in the educational context, it is possible to implement peer-mediated routines to promote appropriate social exchanges with peers that usually are not included in rehabilitative and psycho- educative activities. The intervention comprises:

- for children: specific activities such as speech therapy, music therapy, cognitive activities and emotional and social play (4/6 h per week at the clinical centre)
- for parents: parent involvement into the therapy room (at least 2 h per week) and meetings every 15 days between therapist and parents through video feedback to provide adequate strategies to deal with children with ASD.
- for school: at the beginning, one hour per week with teacher and educator and the child in the school context. Then, meetings every three weeks with school educators in order to share specific interventions' objectives and to organize play activities appropriately.

The focus of the proposed intervention is mainly on building the "net"; in fact, given the pervasiveness of the disorder, the treatment necessarily has to be multimodal, integrated, rooted in the community and it should provide the fundamental involvement of both family and subsequently of school. In order to promote generalization of child's competencies, the network is aimed at providing appropriate strategies to detect and promptly respond to the child's needs, decreasing the child's frustration and boredom.

The intervention is delivered by licensed psychologists after receiving specific training on developmental models of intervention for children with ASD. The team is regularly supervised at least once every three weeks by an expert psychotherapist and all the psychologists have completed the introductory course to the Early Start Denver Model. Further, some of them attended the advanced course.

#### 2.4.2. "As Usual" Intervention

With the term "as usual" intervention, we refer to specific rehabilitative activities such as psychomotricity and speech therapy employed by local community services. In particular, psychomotricity comprises a set of activities to promote communicative and relational abilities by means of body awareness and body movement. Psychomotricity is performed by professionals with a specific bachelor's degree. Moreover, speech therapy directly targets receptive and expressive language without a specific focus on socio-communicative routines. These specific activities represent effective strategies for intervention with preschool children with ASD [46] The intensity is generally from one to three hours per week, calibrated according to child's needs by the reference developmental neuropsychiatrist [46]. In the community services, no active involvement of caregivers and school is provided, but meetings for parents are planned if requested by them and two institutional meetings per year are planned with school educators to monitor the child's schooling.

From the two interventions' description, we would like to underline that the core difference regards the degree of involvement of social context families and school and not the specific rehabilitative activities known to be effective in dealing with children with ASD.

## 3. Results

### *3.1. Analytic Plan*

The data were controlled for normality and homoscedasticity through the Shapiro–Wilk normality test and Levene test for homogeneity of variances. Parametric inferential tests (T test) were used when appropriate to identify group differences before the intervention (T1) and after the intervention (T2), as well as for investigating longitudinal changes. Otherwise, non-parametric tests were performed (Wilcoxon–Mann–Whitney test). Effect sizes were calculated using $r^2$. Linear Regression models were implemented to test for predictors of change, and checked for assumptions. Repeated Measures Analyses of Variance (ANOVA) were performed to check for Group differences. Data were analysed using R statistical software [52].

### *3.2. Preliminary Analysis*

At T1, there were no significant differences in chronological ages between the intervention group (M = 39.76 months; SD = 10.22) and the control group (M = 45.33 months; SD = 8.84), and the time passed between the first and the second assessment was not significantly different between the two groups ($t(35) = 1.26$ ; $p = 0.215$; $r^2 = 0.044$). Further, no significant differences ($t(31) = 1.630$; $p = 0.113$; $r^2 = 0.08$) emerged between the intervention and the control group regarding the socio-economic status of the families.

There were no significant differences at T1 and T2 between the two groups also regarding age equivalents of all the subscales of the Griffiths Mental Development Scales, as well as standardized quotients and the Autism Diagnostic Observation Schedule-Second Edition scores (Tables 2 and 3). Therefore, the whole sample was included to fit linear models. Then, paired T tests in both groups were performed to identify longitudinal changes.

**Table 2.** Developmental quotients in the two groups at T1 and T2.

| | Intervention Group (T1) M (SD) | Intervention Group (T2) M (SD) | INT T1 vs. INT T2 | Control Group (T1) M (SD) | Control Group (T2) M (SD) | CNT T1 vs. CNT T2 |
|---|---|---|---|---|---|---|
| General Quotient | 73.64 (15.84) | 79.12 (22.02) | t(24) = −2.320<br>$p$ = 0.029 *<br>$r^2$ = 0.18 | 69.50 (18.28) | 74.08 (19.51) | t(11) = −1.52<br>$p$ = 0.156<br>$r^2$ = 0.17 |
| Locomotor Quotient | 79.08 (18.54) | 79.68 (19.85) | t(24) = −0.234<br>$p$ = 0.817<br>$r^2$ = 0.002 | 83.50 (21.33) | 76.75 (15.05) | t(11) = 1.924<br>$p$ = 0.081<br>$r^2$ = 0.25 |
| Personal-Social Quotient | 70.36 (21.79) | 75.04 (21.27) | t(24) = −1.52<br>$p$ = 0.142<br>$r^2$ = 0.088 | 64.75 (19.27) | 71.33 (17.19) | t(11) = −1.555<br>$p$ = 0.148<br>$r^2$ = 0.180 |
| Language Quotient | 58.00 (28.97) | 75.32 (35.34) | t(24) = −3.387<br>$p$ = 0.002 **<br>$r^2$ = 0.32 | 60.33 (25.49) | 69.92 (29.70) | t(11) = −2.59<br>$p$ = 0.02 *<br>$r^2$ = 0.38 |
| Eye-Hand Coordination Quotient | 72.80 (18.87) | 78.12 (22.43) | t(24) = −1.77<br>$p$ = 0.089<br>$r^2$ = 0.115 | 64.00 (17.73) | 73.25 (17.32) | t(11) = −2.434<br>$p$ = 0.033 *<br>$r^2$ = 0.350 |
| Performance Quotient | 86.76 (23.38) | 89.40 (24.23) | t(24) = −0.690<br>$p$ = 0.497<br>$r^2$ = 0.019 | 81.33 (27.89) | 85.50 (23.96) | t(11) = −0.791<br>$p$ = 0.446<br>$r^2$ = 0.054 |

* $p < 0.05$; ** $p < 0.01$.

**Table 3.** ADOS scores in the two groups at T1 and T2.

| | Intervention Group (T1) M (SD) | Intervention Group (T2) M (SD) | INT T1 vs. INT T2 | Control Group (T1) M (SD) | Control group (T2) M (SD) | CNT T1 vs. CNT T2 |
|---|---|---|---|---|---|---|
| Social Affect Score | 12.32 (3.18) | 10.04 (3.35) | t(24) = 4.08<br>$p$<0.001 **<br>$r^2$ = 0.41 | 11.75 (3.55) | 10.08 (3.48) | t(11) = 2.80<br>$p$ = 0.017 *<br>$r^2$ = 0.42 |
| Restricted Repetitive Behaviors | 3.88 (1.64) | 3.56 (1.76) | t(24) = 0.902<br>$p$ = 0.376<br>$r^2$ = 0.033 | 3.50 (2.58) | 3.75 (1.76) | t(11) = -0.353<br>$p$ = 0.731<br>$r^2$ = 0.011 |
| Total ADOS-2 Score | 16.20 (4.15) | 13.60 (4.33) | t(24) = 4.40<br>$p$ = 0.0001 ***<br>$r^2$ = 0.46 | 15.42 (5.09) | 13.83 (4.73) | t(11) = 1.73<br>$p$ = 0.112<br>$r^2$ = 0.21 |
| Severity Index | 6.40 (1.63) | 5.84 (1.37) | t(24) = 1.937<br>$p$ = 0.065<br>$r^2$ = 0.135 | 5.92 (1.78) | 5.42 (1.78) | t(11) = 1.483<br>$p$ = 0.166<br>$r^2$ = 0.167 |

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

### *3.3. Longitudinal Changes*

#### 3.3.1. Cognitive Profile

Paired T-tests for dependent samples revealed a significant (t(24) = −2.320; $p$ = 0.029; $r^2$ = 0.18) change in the General Quotient of the Griffiths Mental Development Scales between T1 (M = 73.64; SD = 15.84) and T2 (M = 79.12; SD = 22.02) for the intervention group. Children in the intervention group had a mean difference of 5.48 (SD = 11.81). The control group showed a non-significant (t(11) = −1.52; $p$ = 0.156; $r^2$ = 0.17) longitudinal change between T1 (M = 74.08; SD = 19.5) and T2 (M = 69.50; SD = 18.28) in the General Quotient, with a mean difference of 4.58 (10.43).

Regarding the longitudinal changes for Locomotor, Personal-Social, Performance and Practical Reasoning subscales, no significant differences emerged between the intervention and control groups. However, the control group showed a significant (t(11) = −2.434; $p$ = 0.033; $r^2$ = 0.350) improvement

in the Eye and Hand Coordination subscale between T1 (M = 64.00; SD = 17.73) and T2 (M = 73.25; SD = 17.32). The change between T1 (M = 72.80; SD = 18.87) and T2 (M = 78.12; SD = 22.43) resulted to be non-significant (t(24) = −1.77; $p = 0.089$; $r^2 = 0.115$) in the intervention group.

The Language Quotient showed a significant (t(24) = −3.387; $p = 0.002$; $r^2 = 0.32$) change between T1 (M = 58.00; SD = 28.97) and T2 (M = 75.32; SD = 35.34) in the intervention group with an effect size indicating a strong effect in this subscale. Children in the intervention group had a mean difference of 17.32 (SD = 25.57), showing strong improvements in the Language domain. The difference was significant (t(11) = −2.59; $p = 0.02$; $r^2 = 0.38$) also for the control group, showing a mean difference of 9.58 (SD = 12.82), lower than the intervention group. (Table 2)

#### 3.3.2. Socio-Communicative Profile

A significant (t(24) = 4.50; $p = 0.0001$; $r^2 = 0.46$) difference in the ADOS-2 Total Score emerged between T1 (M = 16.20; SD = 4.15) and T2 (M = 13.60; SD = 4.33) in the intervention group, indicating a strong effect size. The difference was resulted to be non-significant (t(11) = 1.73; $p = 0.112$ $r^2 = 0.21$) in the control group, with a mean difference of -1.58 (SD = 3.18) and a lower effect size. Regarding the intervention group, a significant (t(24) = 4.08; $p < 0.001$; $r^2 = 0.41$) difference in the Social Affect area between T1 (M = 12.32; DS = 3.18) and T2 (M = 10.04; DS = 3.35) emerged, indicating a strong effect and a mean reduction of -2.28 (SD = 2.79). A significant (t(11) = 2.80; $p = 0.017$; $r^2 = 0.42$) difference between T1 (M = 11.75; SD = 3.55) and T2 (M = 10.08; SD = 3.48) was also found in the control group, with a mean difference of −1.67 (SD = 2.06). (Table 3)

### *3.4. Children with and without Intellectual Impairment*

Afterwards, to further investigate trajectories of change, the sample was differentiated in terms of cognitive functioning between the two groups. Coherently with literature and clinical standards, the threshold of 70 was considered in the General Development Quotient of the Griffiths Mental Development Scales. The filter yielded 14 children with General Quotient above 70 in the intervention group (11 children with General Quotient equal to or below 70) and six children above 70 in the control group (six children equal to or below 70).

Regarding the General Quotient, the children without intellectual impairment in the intervention group showed a significant (t(13) = −3.71; $p = 0.003$; $r^2 = 0.51$) longitudinal difference between T1 (M = 84.64; SD = 10.87) and T2 (M = 96.14; SD = 8.69) indicating a strong effect with a mean difference of 11.5 (SD = 11.61). This difference was resulted to be non-significant (t(5) = −1.41; $p = 0.219$; $r^2 = 0.28$) in the control group between T1 (M = 82.83; SD = 6.77) and T2 (M = 87.67; SD = 9.77), with a mean difference of 4.83 (SD = 8.42) and a lower effect size.

Focusing on the Language subscale, children in the intervention group with a General Quotient above 70 at T1 showed a significant (t(13) = −4.00; $p = 0.002$; $r^2 = 0.55$) longitudinal difference between T1 (M = 73.79; SD = 29.54) and T2 (M = 102.14; SD = 17.84), indicating a strong effect with a mean difference of 28.36 (SD = 26.53). Children in the control group who had a General Quotient above 70 showed a non-significant (t(5) = −1.97; $p = 0.106$; $r^2 = 0.44$) difference between T1 and T2 in the Language Quotient. The effect size was still relevant, but the mean difference was 11.67 (SD = 14.50). The difference was resulted to be non-significant between the two groups with respect to children with intellectual impairment.

With respect to the ADOS-2, a significant (t(13) = 4.09; $p = 0.001$; $r^2 = 0.56$) longitudinal difference emerged in the Total Score in the intervention group without intellectual impairment between T1 (M = 11.29; SD = 3.00) and T2 (M = 8.14; SD = 2.60), indicating a strong effect with a mean difference of −3.14 (SD = 2.88). The difference was not significant in the control group of children without intellectual disability (t(5) = 1.6; $p = 0.17$; $r^2 = 0.34$) with a mean difference of −2.67 (SD = 4.08) and a lower effect size.

No significant differences emerged with respect to the Repetitive Restricted Behaviors area in both children with and without intellectual disability.

Furthermore, considering the Social Affect area, a significant longitudinal difference (t(13) = 3.69; $p = 0.003$; $r^2 = 0.51$) emerged for children without intellectual impairment in the intervention group between T1 (M = 11.29; SD = 3.00) and T2 (M = 8.14; SD = 2.60), indicating a strong effect and a mean difference of -3.14 (SD = 3.18). The difference was not significant for children without intellectual impairment in the control group (t(5) = 2.15; $p = 0.08$; $r^2 = 0.48$), with a mean difference of −2.33 (SD = 2.66). With respect to children with intellectual impairment, no significant differences emerged between the two groups.

### *3.5. Predictor Analysis*

In the analysis of predictors of outcomes, all participants were considered without group distinction, given that all children received some form of intervention. Linear Regression Models were fitted in order to test the goodness of different sub quotients at T1 in predicting the General Quotient and the Language Quotient at T2.

The General quotient at T2 was predicted by the combination of Personal-Social ($\beta = 0.46$; $p = 0.006$) and Performance Quotients ($\beta = 0.21$; $p = 0.041$) and the Chronological age ($\beta = -0.60$; $p = 0.003$) at T1. The model was significant ($F(4,32) = 27.38$; $p < 0.001$; Adjusted $R^2 = 0.75$) and explained a significant proportion of the variance. The Language Quotient term resulted to be not significant ($\beta = 0.18$; $p = 0.082$) in this model.

Then, the Language Quotient at T2 was considered as a dependent variable and possible predictors among the subquotients at T1 were investigated. The Language quotient at T2 was predicted by the Language Quotient ($\beta = 0.67$; $p < 0.001$), the Personal-Social Quotient ($\beta = 0.50$; $p = 0.036$) and the Chronological age ($\beta = -1.12$; $p = 0.001$) at T1. The model resulted to be significant ($F(3,33) = 28.74$; $p < 0.001$; Adjusted $R^2 = 0.70$) and explained a significant proportion of the variance.

### *3.6. Responders and Non-Responders*

The 41% of the total sample responded to the interventions with a recovery in the age-equivalent, having a positive learning rate. This group was defined as "responders". In particular, in the intervention group, there was a percentage of 44% of responders, while the control group had a 25% of responders.

To investigate the baseline characteristics of children who positively responded to the intervention, differences at T1 between the responders and non-responders groups were examined.

The General Quotient of the responders group (M = 79.36; SD = 8.85), was significantly (t(35) = −2.12; $p < 0.05$; $r^2 = 0.11$) higher than the General Quotient of the non-responders group (M = 68.00; SD = 18.70) at T1.

Considering the sub quotients, only the Language Quotient of the responders group (M = 69.86; SD = 24.27) was significantly (W = 80; $p = 0.012$) higher than the Language Quotient of the non-responders group (M = 52.00; SD = 27.71) at T1.

Considering the age-equivalents learning rate in the Personal-Social domain, a significant (t(35) = 3.90; $p < 0.001$; $r^2 = 0.30$) difference emerged at T1 in the ADOS-2 score of Repetitive Restricted Behaviors between the responders and non-responders groups. Responders group started with a mean score of 2.31 (SD = 1.49), while the non-responders group had a mean score of 4.54 (SD = 1.74).

Moreover, a significant (t(35) = −2.25; $p < 0.05$; $r^2 = 0.13$) difference emerged at T1 in the Personal-Social Quotient between children who showed improvements in the age-equivalents learning rate of the Language subscale. The responders group started with a mean Personal–Social Quotient of 76.94 (SD = 16.81), while the non-responders group had a mean of 62.14 (SD = 21.80) at T1.

Finally, considering the age-equivalents learning rate in the Performance subscale, children who improved in time (responders) started with a mean Personal-Social Quotient of 77.19 (SD = 16.98), whereas non-responders group had a mean quotient of 61.95 (SD = 21.56) at T1. The difference was significant (t(35) = −2.33; $p < 0.05$; $r^2 = 0.13$).

## 4. Discussion

Given the complexity of evaluating treatment efficacy and the importance of individualized treatment for children with ASD, the main purpose of the present study was to analyse developmental trajectories of preschool children with ASD in order to understand how specific developmental areas evolve in time. As a way to do so, we took into consideration two groups of children exposed to two different kinds of intervention. On the one hand, an intensive intervention focused on the involvement of family with a specific work on wide-range socio communicative abilities and on the other hand, a rehabilitative "as usual" intervention. The results of the empirical research underline how early intensive intervention with parent involvement promotes better results and generalization of a child's competencies [4,5].

Regarding our first aim concerning the differences in the trajectories, our results are in line with the previous literature [5,6,10,22]. In fact, a significant improvement in the general quotient of children exposed to the early intensive intervention emerged, compared to children receiving the rehabilitative "as usual" intervention.

In particular, analysing the specific subscales, it came to light that linguistic-communication abilities present major improvements compared to the other subscales of the general quotient for both groups. In fact, the significantly increased level in the control group is not surprising given that specific rehabilitative activities provided also by local community services improve child linguistic abilities, especially considering both receptive and expressive language. In line with this, the recent literature, using a different measure for investigating the general quotient (Mullen Scales of Early Learning, Communication and Behavior Scales), reported major improvements in linguistic and communicative areas, particularly in both expressive and receptive language after 9 months [45].

Further, our results support the ground idea of developmental models of treatment for ASD that, unlike specific rehabilitative speech therapy-centred treatment, focus on wide-range socio communicative abilities. Developmental models of intervention [4,43] are based on the exploitation of communicative nonverbal behaviours, gestures and their integration together with intentionality and reciprocity to promote the development of language skills through generalization and reduction of avoidance of social interactions. Interestingly, in our intervention group, the mean difference in language skills between the two assessments was greater than the mean difference of the control group. One possible explanation of this result derives from theoretical principles of the intervention that focus on developmental phases with the major aim of promoting intersubjectivity during the exchanges with the other (e.g., supporting non-verbal communication and the correct understanding of social signals). To this end, intentionality and reciprocity are promoted given their importance for language development [40,41]. Further, these results could also be explained by the specific features of the intervention proposed. In fact, the intervention design is aimed to impact the most possible different contexts in the daily living of the child, and greatly extends the possibilities to experiment effective social interplays in a wider range of contexts. In our idea, participating at a major numbers of more appropriate social interactions could lead to better outcomes for children.

From the analysis of the cognitive profile, a significant increase in eye-hand motor coordination for the "as usual" intervention group also emmerged. In fact, a possible explanation could be that rehabilitative interventions such as psychomotricity comprise focused and specific motor activities, involving both gross and fine motor skills. From a clinical point of view of integrating different modalities in order to reach major outcomes, it is important also for networking interventions to comprehend rehabilitative activities to support these aspects.

Concerning the socio-communicative area, that is our second hypothesis, our analysis shows that the general behavioural expressions of ASD decreased significantly in both groups. In fact, some atypical behaviours tended to diminish after the intervention. In particular, children showed improved competencies in the socio-communicative area [9]. These gains were more prominent in the early intensive intervention group, probably thanks to active involvement in the social context that guarantees a generalization of competences. Furthermore, in line with the literature, the area of

restrictive and repetitive behaviours tends to be more stable. In fact, previous studies did not find significant modifications regarding this area after intervention [45,53]. Interventions generally support specific cognitive and social abilities that do not directly impact the area. Furthermore, the specific trends of this domain appear to be under-investigated [54]. However, slight modifications in this area, like the reduction emerged in the intervention group in our results, could be related to the specific work on anxiety reduction, emotions and self-regulatory mechanisms.

Taken together, these results highlight how specific work on a wide range of socio communicative abilities could promote better linguistic gains together with a reduction in symptoms severity with respect to the Social Affect area of the ADOS-2. Interestingly, this area of the ADOS-2 focuses on communicative abilities and social affect, considering different modalities and their integration. These results support the idea that intervention impacts developmental trajectories improving a large spectrum of socio communicative abilities, including receptive and expressive communication but also those important precursors of verbal communication like gestures, imitation and joint attention, fundamental elements to initiate or respond adaptively to the social exchange.

There is great consensus regarding the importance of cognitive level as a prognostic factor considering the developmental trajectory of children with ASD. References [38,39] also pointed out that children with cognitive level equal or above 70 points at the general quotient tend to improve more rapidly over time. In line with this, cognitive abilities are associated with different outcomes. For example, [19] found out that only children without impairment gained significant improvement in adaptive skills after 2 years of treatment, compared to children with intellectual disability. Further, only the first group of children was able to transfer the acquired socio-communication abilities into daily life after 1 year of treatment, showing generalization of competencies. On the contrary, this was not found for children with intellectual impairment. In line with these findings, our results show that children without intellectual impairment in the intervention group reached major gains in the general quotient after intervention. Particularly, the same pattern emerged considering the linguistic quotient, in which children without intellectual disability in the intervention group showed major improvements compared to the other group. With regard to children with cognitive impairment, no differences in both early intensive intervention and "as usual" groups were found.

Another key aspect focusing on developmental trajectories of symptom severity revealed that children without intellectual impairment show a more relevant increase in socio-communicative competencies compared to children with intellectual impairment [38,39]. In line with the analysis considering symptomatology of ASD, our results point out two different trajectories in the group exposed to early intensive intervention with parent involvement: less variability in symptoms expression was found considering children with cognitive impairment, and more gains were found regarding children without intellectual disability. With respect to the group exposed to the early intensive intervention, we found a specific trajectory that characterized children without intellectual impairment: increased level of cognitive abilities, specifically concerning linguistic skills, and reduced levels of symptoms expression. This specific outcome profile was coherent with one specific trajectory defined by [38].

A debate is still open on the identification of pre-treatment variables associated with different response outcomes.

With respect to our third aim, chronological age at the beginning of the intervention had an important role in predicting developmental outcomes, strongly supporting the idea of early intervention with children with ASD. Further, the analysis of pre-treatment variables pointed out the personal–social and performance areas as important predictors of the general quotient after intervention. In our analysis, younger children with better nonverbal intelligence skills, assessed by the Performance subscale, and personal autonomies (assessed by the Personal-Social subscale), showed better developmental outcomes. To our knowledge, no previous studies investigated the relationship between different domains of development and subsequent outcomes. Interestingly,

our results highlighted the association of two specific developmental areas as possible prognostic markers of better developmental trajectories.

A wide consensus is present concerning chronological age, supporting early intervention [6,23]. However, the relation with cognitive functioning appears to be more complex, with controversial evidence. On the one hand, lower cognitive skills are found to be associated with larger improvements [55], pointing out the possibility of substantial improvements for children starting below the average. On the other hand, other authors found out that higher cognitive skills predicted better outcomes on child developmental trajectory [39,56], suggesting a complex relationship that needed to be further investigated. More interestingly, sub-components of the general intelligence were investigated to identify markers in the neurodevelopmental profile and early neurodevelopmental milestones that could predict later cognitive functioning and the acquisition of language [40].

With the aim to deeply analyze developmental domains and given the significant improvement concerning language skills in our results, we focused the analysis on the Language Quotient after intervention, showing that pre-treatment language skills and personal-social abilities, together with age, predict better linguistic outcomes. This could underlie how, in the development of language, an important role is played by nonverbal communicative aspects [57]. In fact, the Personal–Social subscale investigates the development of a wide range of nonverbal communicative and social signals (e.g., social smile, showing, orienting the others' gaze and communicative gestures) whereas in the Language subscale, besides the verbal skills, another set of communicative behaviours (e.g., pointing) are investigated, supporting the idea that the association between these two factors could represent possible prognostic markers specific for language development.

Taken together, and in line with other recent research works [40], these results seem to support the impact of wide-range of socio communicative behaviours and skills on developmental trajectories, regarding both the general cognitive skills and, more specifically, on language development [58]. Further, despite previous research depicting the role of symptom severity on intervention outcome, our analysis suggests that developmental areas were more predictive of outcomes than symptom severity before the intervention [26,40].

On the basis of these results, the analysis of responders focused on differentiating children who recovered in age-equivalents, narrowing the gap between their chronological and mental age, from children who seem to remain more stable. Interestingly, the responders showed a higher cognitive functioning before intervention and, in particular, greater language skills, coherently with our previous results. Furthermore, children who narrow the linguistic gap started with higher personal–social abilities and, interestingly, children who closed the performance gap also started with higher personal–social abilities. These results highlight the role of some cognitive factors (in particular, personal–social skills) not only in predicting outcomes after intervention but also in differentiating children who showed significant recovery from those characterized by more stable response trajectories.

Finally, concerning the trajectory of symptoms severity, our results evidence a significantly higher proportion of children who showed a reduction in symptomatology in the intervention group. Unexpectedly, a significant difference in restricted and repetitive behavioural pattern before intervention emerged between children who show a better recovery in personal–social skills, being characterized by lower symptom severity, and children who show a more stable outcome in this cognitive domain. This result may point out a potential role of this area in supporting or impeding the development of personal–social abilities and require further investigation in order to better understand its impact on the developmental trajectory.

This knowledge may have important implications for clinical practice, providing clinicians more information about specific areas to be targeted by the intervention and disclosing the importance of specific behaviours for subsequent language outcomes.

*Limitations*

This study presents some limitations. First of all, despite our results being in line with previous literature, a main limitation of the present work is represented by the small sample size, and hence, results should be replicated in studies with larger samples. Further, sample size is important with respect to the high variability reported in the literature concerning different response trajectories. A small sample size reduces the possibility to investigate clusters of response profiles [39]. Moreover, the sample is unbalanced with respect to gender, thus reducing the possibility to investigate gender differences in the response trajectory, as emerged by recent literature [59]. In addition, our sample was not randomized. However, our aim was to understand intervention outcomes guarantying to patient better opportunities with respect to the specific intervention offered by the local territories. Children were assessed by independent examiners that were aware of their local origin but blinded to this study and not involved in children's therapeutic intercourse. The presence of only two assessments represents a limitation in order to better evaluate the response trajectory. Thus, an additional point to address in our further studies will be to measure children's developmental profiles in other time points in order to trace the response during time evidencing improvements and tendencies towards the stabilization of the profiles. Another future perspective is represented by a detailed analysis of specific socio-communicative elements evaluated by the ADOS-2. As an example, social affect behaviours such as pointing, showing and quality of social overtures could be important markers of change to be investigated, as pointed out by some research results [58], and could play a role in the response. Finally, characterizing children who narrow the gap and those displaying more stable trajectories could better inform about prognostic markers associated with better outcomes. In addition, it could disclose new features to be taken into account in order to explain the variability in the response and improve developmental outcomes of more persistent profiles.

## 5. Conclusions

Identifying early trajectories of children with ASD has both theoretical and clinical implications. From a theoretical perspective, it can inform our understanding of early predictors and mediators of change in order to identify specific elements to be targeted in the intervention design. Further, this type of perspective enhances knowledge about ASD according to a developmental perspective.

From a clinical standpoint, careful attention to developmental trajectories may help in structuring individualized intervention based on a child's specific competencies in every phase of development. Finally, it is important to emphasize the fundamental role of social context in order to guarantee generalization of child competencies and better outcomes over time.

To conclude, the importance of networking intervention on child cognitive and social development led us to exploit online technologies in order to support social context through regular meetings to build up a valid online network.

**Author Contributions:** Conceptualization and Methodology: P.V., A.B. Formal Analysis: G.B. Patient recruitment: A.B. Data Curation: G.B., S.P. Writing—Original Draft Preparation: G.B., S.P., A.B. Writing—Review and Editing: P.V., G.B., S.P., A.B. Supervision: P.V. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Acknowledgments:** We gratefully acknowledge the families participating in our research and all the clinical psychologists and psychotherapists of ODFLAB.

**Conflicts of Interest:** The authors declare no conflict of interest.

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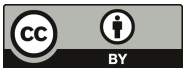

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Article

# The Source of Palm Orientation Errors in the Signing of Children with ASD: Imitative, Motoric, or Both?

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Received: 20 February 2020; Accepted: 28 April 2020; Published: 30 April 2020

**Abstract:** Palm orientation reversal errors (e.g., producing the 'bye-bye' gesture with palm facing inward rather than outward as is customary in American culture) have been documented in the signing of deaf and hearing children with autism spectrum disorder (ASD) and in the imitation of gestures by signing and non-signing children with ASD. However the source of these unusual errors remains opaque. Given that children with ASD have documented difficulties with both imitation and motor skills, it is important to clarify the nature of these errors. Here we present a longitudinal case study of a single child with ASD, a hearing, signing child of Deaf parents. Samples of the child's signing were analyzed at ages 4;11, 6;2, 10;2, and 14;11. Lexical signs and fingerspelled letters were coded for the four parameters of sign articulation (handshape, location, movement, and palm orientation). Errors decreased for handshape, location, and movement after age 4;11, but increased on palm orientation from 4;11 and remained high, exceeding 55% of signs by 14;11. Fingerspelled letters contained a large proportion of 180-degree reversals, which suggest an origin in imitation differences, as well as midline-facing errors, suggestive of a motor origin. These longitudinal data suggest that palm orientation errors could be rooted in both imitation differences and motoric difficulties.

**Keywords:** autism spectrum disorder; sign language; imitation; cognition; language acquisition

## 1. Introduction

We previously presented the first report [1] on an aspect of the language development of native-exposed signing children with autism spectrum disorder (ASD). In that paper, we showed that three young children with ASD who had been exposed to American Sign Language (ASL) from birth by their deaf parents exhibited an unusual formational pattern in their signing: the reversal of the palm orientation parameter, such that signs normally produced with an outward-facing palm were produced with an inward-facing palm, or vice versa. Since such errors are not known to occur in the typical development of ASL beyond an early age, we speculated that such reversals could be unique to signing children with ASD and as such might be included in clinical criteria adapted for sign-exposed children. Interestingly, to-date signing children with ASD have not been found to produce pronoun reversals [2] like those characteristically found in the speech of some hearing children with ASD [3–5] as well as very young typically-developing hearing children [6–8], raising the possibility that the documented palm reversals could be a sign language analog to pronoun reversals in speech—that is, errors that occur due to the child's difficulty understanding how linguistic forms shift between speakers/signers.

These palm reversal errors have thus provided an opportunity to speculate about the kinds of cognitive, linguistic, or motoric differences that might underlie their production by signers with ASD. At the time of our initial report, we followed the interpretation used in a review of 21 studies of imitation by children with ASD [9] which described difficulties with "self-other mapping", the translation of others' movements onto one's own body. Particularly strong evidence of this interpretation came

from a number of studies [10–13] which had found reversal errors in gesture imitation by hearing children with ASD that were of the same type as those we later documented in signing children with ASD. Although the errors we previously reported [1] were not errors of imitation, but rather of sign production, both elicited and spontaneous, we found it plausible that differences in imitation style could contribute to erroneous phonological representations of signs, thus accounting for the reversed palm orientation parameter in sign language production. We later elaborated on this hypothesis [14], describing a "visual matching strategy" in imitation that is characteristic of some learners with ASD, in which signs are imitated as they appear from one's own perspective, resulting in palm orientation reversals and other erroneous sign productions, such as reversals of the direction of movement.

Despite the reasonable conjecture that such errors could be the result of an imitation difference, motor issues cannot be excluded as a possible cause of palm orientation errors. From 50 to 80% of children with ASD exhibit motor impairments [15–18], including basic motor skill deficits in reaching and walking [19,20], gross and fine motor incoordination [15,17,21], as well as deficits in praxis/motor planning [22–26], and such deficits have been found to extend to deaf, signing children with ASD [27]. Children with motor issues with the articulation of signs might execute signs with the palm facing the midline of the body, which is the default resting position of the palm when the arms are hanging at one's sides. Producing inward- or outward-facing palm orientations requires the supination and pronation of the forearm, respectively. The ability to pronate and supinate the forearm develops throughout early childhood, with about 90% of typical children reaching mastery by age 6.5 [28]. Signers with motor disorders resulting from Parkinson's Disease have been shown to neutralize the palm orientation parameter by producing signs toward the midline rather than with inward or outward orientation as a result of reduced motoric effort [29].

Given that children with ASD have documented difficulties with both imitation and motor skills, it is important to clarify the nature of the unusual sign articulation errors that we have documented in signing children with ASD. In particular, longitudinal data on the developmental trajectory of such errors in comparison with the other articulatory parameters of sign could be illuminating. In this regard it is possible to make predictions about what the developmental trajectory of articulatory parameters would look like under two hypotheses:

*Motor origin hypothesis*: Motor difficulties are predicted to result in the palm facing the midline (default resting position) rather than outward or inward (pronated or supinated). Furthermore, if motor issues are the sole or primary cause of palm orientation errors, then the error rate in palm orientation is predicted to: (a) mirror that of the other sign language parameters (handshape, location, and movement) and (b) decrease over time as motor skills improve.

*Imitation hypothesis*: Differences in imitation are predicted to result in 180-degree reversal errors (signs specified for outward orientation produced with inward-facing orientation and vice versa); see Figure 1. Furthermore, if differences in imitation are the sole or primary cause of palm orientation errors, then the error rate in palm orientation is predicted to: (a) diverge from that of the other articulatory parameters (which are less affected by the visual matching imitation style), and (b) could remain relatively stable over time, as imitations solidify into mental (phonological) representations.

(**a**) (**b**)

**Figure 1.** (**a**) Example of how the fingerspelled letter T is typically produced; and (**b**) How the fingerspelled letter T would be imitated with 180-degree reversal.

A large number of the palm orientation reversal errors documented in our prior report [1] were produced on fingerspelled letters rather than on lexical signs: we reported 50 reversal errors on fingerspelled letters and five reversal errors on lexical signs. Fingerspelling is a system whereby the written alphabet of a spoken language is represented by different hand configurations. The fingerspelling system in ASL is one-handed; that is, each letter of the written alphabet is represented by a unique hand configuration (see Appendix A). Signed languages differ from each other in how they represent written alphabets as well as in the extent to which fingerspelling plays a role in the larger signed language. It is conventionally understood that the American Deaf community employs fingerspelling to a greater extent than in most other Deaf communities around the world [30].

Fingerspelling is most often used for proper names or for technical or novel terms for which a conventional lexical sign is lacking. Unlike lexical signs, which only employ one or two different handshapes [30], fingerspelling requires the signer to execute a series of different handshapes, one for each letter of the word being spelled. Lexical signs can be specified for different locations from the head to the waist or can be made in neutral space (e.g., the sign MOTHER on the chin, the sign FATHER on the forehead), [Links to video examples from the SignBank database [31] are provided for all lexical signs in the online version of the paper.] In contrast, fingerspelling in ASL is performed in a relatively small neutral space in front of the signer's torso. Most fingerspelled letters are static handshapes without movement, with the exceptions of the letters J and Z (Appendix A), while lexical signs draw from an extensive set of possible movements. Finally, while palm orientations of lexical signs can be specified to face up, down, toward the midline, to the sides, or toward or away from the signer's body, fingerspelled letters in ASL all face outward from the signer's body with a pronated forearm, with the exception of the letters G, H, P, and Q. The letters G and H face inward, with the forearm rotated inward (supinated), while the letters P and Q face downward, with a flexed wrist and pronated forearm (Appendix A), though note that there is a variant production of P with only very slight flexion of the wrist and supination of the forearm, resulting in inward palm orientation [32], but the participant in this study did not produce any tokens of this variant.

In the sections that follow we distinguish between lexical signs and fingerspelled letters and analyze them separately. We do so for the following reasons: (1) we observed a large number of fingerspelling errors in our previous work [1]; and fingerspelled words (2) require the execution of a series of hand configurations in sequence; (3) are uncomplicated by changes in location; (4) are largely

uncomplicated by changes in movement; and (5) present frequent opportunities for 180-degree palm reversals given their specification for outward-facing palm orientations.

This study presents a longitudinal case study of a single native signer with ASD, a hearing son of two Deaf parents, and analyzes the four articulatory parameters of his signs over a 10-year period, in order to shed light on the nature of palm orientation errors in ASD.

## 2. Materials and Methods

The parents of the participant gave their informed consent before including their child in the study. The study was conducted in accordance with the Declaration of Helsinki, and procedures were prospectively approved by the Institutional Review Board of the University of Texas at Austin (at ages 4;11 and 6;6; Protocol #2007-08-0022), Boston University (age 10;2; Protocol 2471E) and Miami University (age 14;11; Protocol 01375).

### *2.1. Participant*

The child described here was one of the three natively sign-exposed children with ASD described previously [1]; in that work he was referred to as "Child 3". He is a left-handed hearing male, diagnosed with ASD at age 2;6 by a licensed clinical psychologist. He has two Deaf parents who communicate primarily through ASL and a younger hearing brother. His parents indicated that he has received occupational therapy for low muscle tone affecting his fine motor skills. (While handedness is certainly a relevant factor in considering how children might imitate signs [14,33], the palm orientation parameter is unaffected by handedness. Therefore the child's left-handedness is not considered further in our analyses.)

In addition to the data collected at age 6;6 reported previously [1], we visited the child at three different times over the course of ten years: at ages 4;11, 10;2, and 14;11. Over the course of those ten years we collected a number of standardized measures of language (both English and ASL), nonverbal intelligence, and ASD; these are reported below. He exhibits moderate ASD symptoms, by behavioral observation (ADOS-2) and by parent report (SCQ and AQ-Adolescent). He scores in the impaired range on nonverbal intelligence (TONI-4) and on receptive language for English (CELF-5; PPVT-4) and ASL (ASL RST).

#### 2.1.1. Autism

The Autism Diagnostic Observational Schedule, Second Edition (ADOS-2; [34]) was administered at age 9;11 by a clinician who had attained research reliability on the instrument and was fluent in English and ASL. The child's total score of 15 (corresponding to a severity score of 6 on a scale of 1–10) indicated moderate ASD symptoms and was above threshold for autism classification. His mother completed the Social Communication Questionnaire (SCQ; [35]), Lifetime Form, at 10;2 and 14;11; his total score was above threshold for ASD risk at both ages (raw score of 14 at 10;2 and raw score of 16 at 14;11). Additionally, his mother completed the Autism Quotient (AQ; [36]), Adolescent Version, at 14;11. His score of 34 was above the threshold score for ASD of 32.

#### 2.1.2. Intelligence

We administered the Test of Nonverbal Intelligence, Fourth Edition (TONI-4; [37]) at age 10;2 and 14;11. At 10;2 he achieved a raw score of 6, which translates into a standard score of 69, just under 2 SD below the mean. At 14;11 he achieved a raw score of 25, corresponding to a standard score of 86, or 17th percentile for his age and an age equivalent of 9;0.

#### 2.1.3. Language

Our participant is the bimodal bilingual child of Deaf parents. It is important to note that there is no established profile for bimodal bilingual children exposed to both a signed language and a spoken

language, such as the hearing children of Deaf adults [38]. However, hearing children of Deaf adults typically have speech that is equivalent to monolingual hearing children by about age 7 [39]. At age 6;6 our participant's mother filled out the Language Proficiency Profile, Second Edition (LPP-2; [40]), a parent report measure to estimate global communication skills. His total score of 26 indicated language well below his chronological age. At age 10;2 we administered both the Peabody Picture Vocabulary Test, Fourth Edition (PPVT-4; [41]) and the American Sign Language Receptive Skills Test (ASL RST; [42]) to obtain measures of his receptive skills in English and ASL. He obtained a standard score of 46 on the PPVT-4 (1st percentile), corresponding to an age equivalent of 4;1. On the ASL RST, he obtained a raw score of 6, corresponding to an age equivalent of under age 3, the youngest age for which norms on this test are given. At 14;11 we repeated the ASL RST and added the Receptive Language Index subtests of the Clinical Evaluation of Language Fundamentals, Fifth Edition (CELF-5; [43]). On the ASL RST he achieved a raw score of 12, corresponding to a standard score of 71, about 2 SD below the mean. On the CELF-5, he achieved a standard score of 45, more than 2 SD below the mean.

#### 2.1.4. Prior report

In our previous report [1], we described data collected from the child when he was age 6;6. At 6;6, the child produced 59 signs, of which 35 (59.3%) contained one or more articulatory errors. For a summary of the child's articulation errors at that age, see Table 1.

**Table 1.** Articulation errors previously reported at age 6;6.

| Parameter | Number of Errors | Description of Errors |
|---|---|---|
| Location | 3 | On the sign ORANGE, he failed to raise his hand from the resting position in his lap and therefore made the sign in contact with his knee rather than his chin (confirmed by maternal repetition immediately afterward) and produced the sign ICE-CREAM in neutral space rather than at the chin. Finally, he produced the sign STAR without contact between the hands, at chest level rather than chin/head level |
| Handshape | 9 | He produced a 4-handshape instead of an H-dot handshape (i.e., a handshape with the first and second fingers extended and together, third and fourth fingers closed, and thumb extended) on RABBIT, a 5-handshape instead of a V-handshape on DANCE (4 tokens), a baby-C-handshape instead of a G-handshape on the sign GREEN, an A-handshape instead of an X-handshape on the sign APPLE, an 8-handshape instead of a G-handshape on the sign CHICKEN, and a 5-handshape instead of an H-dot handshape on the sign HORSE |
| Movement | 23 | Forward movement (outward) rather than inward on the sign LION (two tokens) and COMPUTER (one token). He did not execute a path movement on several signs that normally exhibit path movement (such as ELEPHANT and GIRAFFE), and reduced movement on several signs that typically exhibit repeated cycles of movement (e.g., HORSE, DUCK, MONKEY, BEAR and CHICKEN). He deleted the movement segment entirely on the sign ICE-CREAM. Other simplifications included the loss of the non-dominant hand on the sign DANCE as well as the dropping of one hand from a two-handed sign (BEAR, MONKEY). Several signs exhibited wild, uncontrolled movement, which were only interpretable because the parent repeated the sign with the correct form; these included DANCE and BLUE. Finally, he produced the sign YES with a forearm rotation rather than with a nodding movement of the wrist (two tokens) |
| Palm orientation | 4 | Two substitutions of an inward orientation for an outward orientation (on the sign FLASHING-LIGHT as well as a wave gesture) and the substitution of a downward orientation for a midline-facing orientation (TURTLE) and for an inward orientation (THREE) |

### *2.2. Procedure*

The child was observed at home at all four time points. At age 4;11, he was observed in an unstructured, naturalistic interaction with his Deaf father, who engaged with him while reading to him from a picture book. At 6;6 and 10;2, he was observed interacting with the first author, a hearing researcher fluent in ASL, who performed a series of experimental tasks, including eliciting fingerspelled words and lexical signs. At 14;11, he was observed interacting with his Deaf mother, who asked him a series of questions in ASL about friends, school, books, and movies.

### *2.3. Coding*

Using ELAN (EUDICO Linguistic Annotator) multimodal coding software [44], we coded 12 continuous minutes from each time point (ages 4;11, 6;6, 10;2, and 14;11) for all signs produced. For age 6;6, previously reported [1], we coded a new 12-minute span of video. Each letter of a fingerspelled word was coded and counted as an individual sign. Each sign was coded for handshape, location, movement, and palm orientation (inward, outward, upward, downward, or midline-facing). The coded value for each parameter was scored as being produced correctly or as an error based on standard citation forms; we used the ASL Signbank as a reference (see https://aslsignbank.haskins.yale.edu/) [31]. Where movement segments were deleted, resulting in a missing second location, errors were coded as movement errors only. Errors were qualitatively described so as to allow for further analysis.

### *2.4. Reliability*

To ensure the reliability of the coding system, two 5-minute segments (one from age 4;11 and one from 14;11) were blindly recoded by a second trained coder experienced in the coding of ASL. Differences in coding were discussed by both coders and disagreements were resolved through consensus. The main coder then adjusted the rest of the coding to reflect the decisions made through consensus discussion with the second coder.

## 3. Results

Table 2 presents a comparison of the overall number of signs produced and the number of signs produced per minute. Overall sign production increased over time, although note that we have counted individual fingerspelled letters as separate signs. Importantly, the child's fingerspelling increased over time, which could account for the greater number of signs produced, especially at 14;11. This increase in fingerspelling is in line with other reports of the developmental trajectory of fingerspelling, which have shown that fingerspelling to children by adults increases as the children mature, and the fingerspelling produced by such children increases in turn [30].

**Table 2.** Comparison of quantity of signs produced and error rates across time points

| Age | 4;11 | 6;6 | 10;2 | 14;11 |
|---|---|---|---|---|
| Number of sign tokens produced | 76 | 124 | 108 | 197 |
| Sign tokens/min | 6.33 | 10.33 | 9.0 | 16.42 |

Table 3 presents the total number of lexical signs and fingerspelled letters produced at each time point, and the number of errors on each of the four sign parameters produced for both types of signs. Note that fingerspelled letters are all produced in neutral space and generally do not exhibit movements, except for the letters ɉ and z (see Appendix A); thus location and movement errors are unlikely on fingerspelled letters.

**Table 3.** Errors on lexical signs and fingerspelled letters at each age, classified by parameter.

| AGE | 4;11 | | 6;6 | | 10;2 | | 14;11 | |
|---|---|---|---|---|---|---|---|---|
| | Lexical (*N* = 72) | Fingerspelling (*N* = 4) | Lexical (*N* = 69) | Fingerspelling (*N* = 55) | Lexical (*N* = 43) | Fingerspelling (*N* = 65) | Lexical (*N*= 76) | Fingerspelling (*N* = 121) |
| Handshape error | 15 (20.8%) | 0 | 5 (7.2%) | 0 | 0 | 0 | 2 (2.6%) | 0 |
| Location error | 5 (6.9%) | 0 | 2 (2.9%) | 0 | 0 | 0 | 3 (3.9%) | 0 |
| Movement error | 13 (18.1%) | 0 | 8 (11.6%) | 0 | 1 (2.3%) | 0 | 9 (11.8%) | 0 |
| Palm orientation error | 14 19.4%) | 0 | 5 (7.2%) | 30 (54.5%) | 2 (4.7%) | 19 (29.2%) | 2 (2.6%) | 110 (90.9%) |

Figure 2 shows the child's error rates on the four sign articulation parameters across the four time points, collapsing lexical signs and fingerspelled letters. Error rates for handshape, location, and movement decrease over time, while the palm orientation parameter shows the opposite trend, increasing to an error rate of over 50% at age 14;11. By comparison, studies of the early acquisition of phonological parameters in ASL have found that the handshape parameter is the most error-prone early in development, while location is acquired earliest, as appears to be the case for this participant at age 4;11. Most studies of phonological development in ASL have primarily focused on children who are much younger than the participant in this study, i.e., under age 2 [45–47].

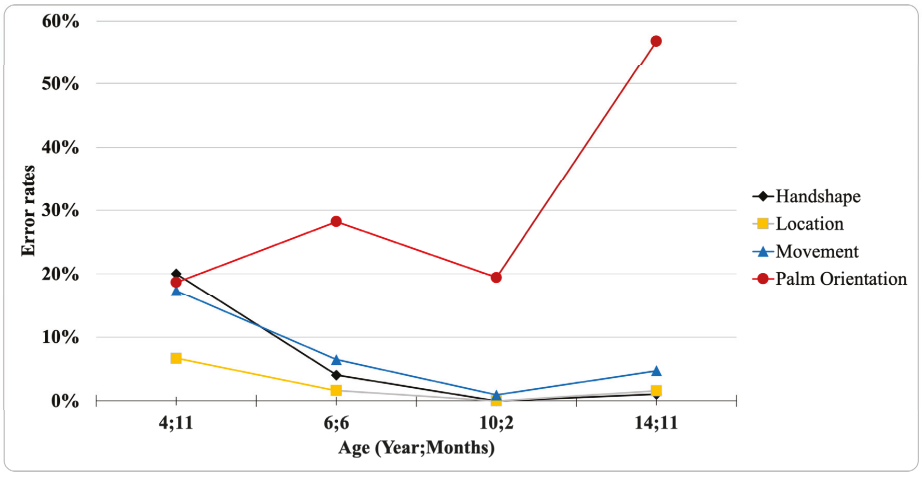


**Figure 2.** Proportion of signs exhibiting errors in the four sign parameters at four ages.

We distinguished three different types of palm orientation errors: 180-degree reversals (substitutions of inward palm orientation for outward or vice versa), midline errors (neutralization of the palm orientation parameter such that the palm faced toward the midline rather than inward, outward, up, or down), and other errors (e.g., substitutions of an upward- or downward-facing palm for inward or outward). Table 4 reports the frequency of each error type of error at each age.

**Table 4.** Palm orientation errors by type at each age.

| Error Type | 4;11 | 6;6 | 10;2 | 14;11 | Total |
|---|---|---|---|---|---|
| 180-degree reversal errors | 1 (7.1%) | 15 (42.9%) | 8 (36.4%) | 58 (50.9%) | **82 (45.1%)** |
| Midline errors | 2 (14.3%) | 18 (51.4%) | 13 (59.1%) | 54 (47.4%) | **87 (47.8%)** |
| Other errors | 11 (78.6%) | 2 (5.7%) | 0 | 0 | **13 (7.1%)** |
| **Total** | **14** | **35** | **21** | **112** | **182** |

Looking across all of the palm orientation errors produced in our sample, 159 of 182 errors (87.3%) were produced on fingerspelled letters while the remaining 23 errors (12.6%) were produced on lexical signs. We report all fingerspelled letters in Table 5 below. Note that a number of fingerspelled names produced at age 14;11 were redacted to protect the participant's identity. In these redacted fingerspelled names, the participant produced 7 names: four 5-letter names and one 4-letter name with all letters produced facing the midline; one 4-letter name with all letters reversed, and one 4-letter name with the first three letters reversed and the last letter produced with correct outward orientation. There were no instances of G, H, P, or Q in these names, so the target orientation for all letters was outward.

It is clear from Table 5 that the child produced fingerspelled letters with inconsistent palm orientation. Indeed, he produced certain fingerspelled handshapes with different palm orientations during the same session (e.g., with in/mid/outward palm orientation on E and O at 6;6 and D, A, and Y at 14;11) and varied the palm orientation of fingerspelled letters at different ages (e.g., L outward at 10;2 but inward and midline-facing at 14;11).

In order to understand the inconsistency exhibited in palm orientation, we examined how palm orientation errors occurred within fingerspelled words. First, some words maintained a consistent palm orientation, be it correct (outward) as in #DOOR. at 6;6, or incorrect such as the midline orientation exhibited in #SWING at 14;11 or reversed (inward) as in the #YODA example illustrated in Figure 3. [As is conventional in the literature, fingerspelled words are denoted by a preceding pound sign (#)]. However, we also found instances in which the child switched between (correct) outward palm orientation and (incorrect) inward palm orientation within the same fingerspelled word. Words that follow this pattern of inconsistency include #TEACH, #PHONE, #MOTHER, and #FATHER (Figure 4) at 10;2. Recall that all fingerspelled letters are typically produced with the palm facing outwards (with pronated forearm) except for G and H (which face inward with supinated forearm), and P and Q (which face downward, with pronated forearm and flexed wrist). This difference in specification for palm orientation means that in words that contain these four letters, the signer must switch between outward, inward, and downward palm orientations in the course of normal signing, which requires the pronation, supination and re-pronation of the forearm (as well as wrist flexion for P and Q). The child in this study also produced words without errors in which he successfully switched between inward, outward, and downward palm orientations, such as the word #TELEPHONE at 6;6, where P and H were produced with correct downward and inward orientations, respectively, and all other letters with correct outward orientation (though note the substitution of I for L). Other examples of this include the words #SCHOOL (produced without the C), #GIRL, #CHAIR, and #BUG produced at 10;2. However there are also examples in which the child produced a reversed palm orientation on letters that are adjacent to H. Examples that follow this pattern include #CHAIR at 6;6, #TEACH, #PHONE, #MOTHER, and #FATHER (Figure 4) at 10;2, and #SCHOOL and #THEINCREDIBLES at 14;11.

**Table 5.** Fingerspelled letters produced at each age. Letters produced with correct outward palm orientation (all letters except G, H) are represented in plain font, letters produced with correct inward palm orientation (G, H) are underlined, while letters that exhibited 180-degree reversals are bolded and letters produced with midline errors are italicized. Some tokens contain spelling errors produced by the child, e.g., the substitution of I for L.

| | **4;11** | **6;6** | **10;2** | **14;11** |
|---|---|---|---|---|
| | R | W | B-A-L-L | **S**-*C*-H |
| | B | F | P-A-P-E-R | **S-C**-H-**O-O-L** |
| | D-W | V | G-I-R-L | *S-W-I-N-G* |
| | | **B-O-O-K** | S-H-O-O-L | [redacted] |
| | | D-O-O-R | B-I-R-D | [redacted] |
| | | **C**-H-**A-I-R** | T-E-*A*-**C**-H | [redacted] |
| | | *W-A-T-C-H* | **P**-P-**H-O-N-E** | Y |
| | | *S-H-O-E-S* | *D-E*-S-K | [redacted] |
| | | *T-A-B-I-E* | *C*-H-*A*-I-R | A |
| | | *C-A-P* | *D-O-L-L* | *D-I-D* |
| | | **B-E-D** | F-*A*-T-H-E-R | *B-E-D-A* |
| | | S-C-I-S-S-O-R-S | M-*O*-**T**-H-**E**-R | [redacted] |
| | | T-E-I-E-P-H-O-N-E | W-V-A-N | [redacted] |
| | | **K** | B-U-G | *B-E* |
| | | | | **D-L**-F-**W** |
| | | | | M-D |
| | | | | *N-A-D-D-W* |
| | | | | M-D |
| | | | | **P-A-R-K** |
| | | | | **P-A-R-I-S** |
| | | | | C |
| | | | | [redacted] |
| | | | | **D-A**-*S*-**R** |
| | | | | **D-A** |
| | | | | **T-H-E-I-N-C-R-E-D-I-B-L-E-S** |
| | | | | **A-R-L** |
| | | | | **P-E-T-E-R**-R |
| | | | | R |
| | | | | **R** |
| | | | | **D** |
| | | | | **Y-O-D-A** |
| Total number of fingerspelled letters produced | 4 | 55 | 65 | 121 |
| Total number of midline errors | 0 | 18 (32.7%) | 11 (16.9%) | 53 (43.8%) |
| Total number of reversed letters | 0 | 12 (21.8%) | 8 (12.3%) | 57 (47.1%) |

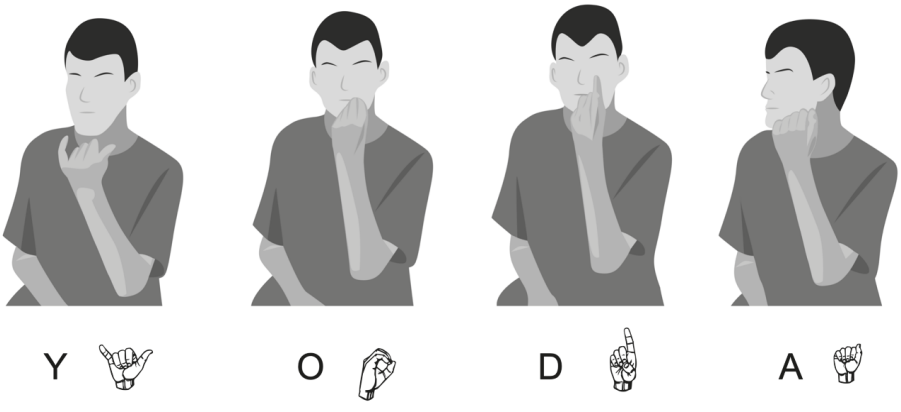


**Figure 3.** The fingerspelled word #YODA produced with reversed, inward palm orientation on each handshape at 14;11. The word was produced rapidly and fluently, unlike the labored production of #FATHER in Figure 4.


**Figure 4.** The fingerspelled word #FATHER produced on the left hand at 10;2 with correct outward palm orientation on the letter F, mid-facing orientation on the letter A, correct outward palm orientation on the letter T, correct inward orientation on the letter H, incorrect reversed palm orientation on the letter E, and correct outward palm orientation on the letter R. Note the lack of inhibition of movement of the non-signing right hand, indicative of a lack of motor control.

## 4. Discussion

We have documented the development of the four parameters of sign articulation over a period of ten years in a single child with ASD, a natively sign-exposed hearing child of two Deaf parents. This is the first time that the sign development of a native signer with ASD has been studied longitudinally. We had hypothesized that the palm orientation errors documented previously [1] could have imitative or motoric origins, and that the developmental trajectory of the palm orientation parameter, in comparison with the other parameters of sign language development, could shed light on this question. Here we evaluate the evidence for both hypotheses.

Is there evidence in favor of the motor origin hypothesis? Yes. The strongest evidence is the occurrence of palm orientation errors produced toward the midline rather than inward or outward. Such errors accounted for 47.8% of the palm orientation errors in our sample, occurred at all ages studied, and reflect the neutralization of the palm orientation parameter toward a default resting position [29]. The fact that errors on the three other parameters (handshape, location, and movement) decrease over time suggests a developmental trajectory of improvement in motor skills that does not extend to palm orientation; in particular, the handshape parameter, which has the highest error rate at age 4;11, decreases nearly to zero by 10;2, and remains stable at 14;11. Numerous studies have found that, of the three major parameters of handshape, location, and movement, handshape is the parameter that is mastered latest in typical development [45,46,48–53], probably due to the late development of the fine motor control required to produce handshapes accurately (though note that not all of these studies examined palm orientation as a separate parameter).

A second source of evidence in favor of the motor hypothesis is the fact that the child sometimes reversed palm orientation on letters that were adjacent to the inward-facing letters G and H. This suggests that the child anticipated the switch in palm orientation on a subsequent letter (as in the C in #CHAIR at 6;6 or the T in #MOTHER at 10;2), or failed to reorient his palm to face outward (i.e., re-pronate the forearm) following one of the inward facing letters (as in the O in #PHONE, the E in #FATHER, and the E in #MOTHER at 10;2). Additional evidence of motor control issues include the lack of inhibition of the non-dominant hand shown in Figure 4, and the unusually high signing shown in Figures 3 and 4.

Given the evidence for motor impairment causing palm orientation errors, is there also evidence in favor of the imitation hypothesis? Here, too, the answer appears to be yes. Unlike the other parameters of sign formation (handshape, location, and movement), which show a clear decrease in error rate over the ten-year period, palm orientation errors increase over time, to above 50% at age 14;11, and reversal errors made up nearly half of all palm orientation errors documented in this study (82 out of 182 errors; 45.1%). Particularly striking are fingerspelled words that do not include the letters G and H but which were produced with consistently inward palm orientation, as in #PARK, #PARIS, and #YODA (Figure 3) at 14;11. It is unlikely that such 180-degree reversal errors would result from motoric difficulties, since the supination of the forearm entailed in the production of inward palm orientations is as motorically difficult to execute as the pronation of the forearm entailed in outward palm orientations. Instead, these reversal errors are suggestive of differences in imitation during the sign learning process in which the child reproduces what he sees from his perspective ("visual matching"), yielding forms with reversed palm orientation. It is worth noting that most of the 180-degree reversal errors described here involve the substitution of an inward-facing palm orientation (supination) for an outward-facing palm orientation (pronation); 75 of the 82 (91.5%) 180-degree reversal errors described here fall into this category. We believe that this finding is again due to the fact that nearly all fingerspelled letters are typically produced with an outward-facing palm, and the child reported on here tended to reverse palm orientation on fingerspelled letters. Despite this trend, a minority of errors (7 of 82 or 8.5%) involved the substitution of an outward-facing palm for an inward-facing palm, showing that reversal errors can replace inward with outward palm orientations as well as outward with inward palm orientations. Other errors of this type, such as the production of the lexical sign BUTTERFLY with outward-facing rather than inward-facing palms, have been reported before [33]. More study is warranted to better understand which lexical signs could be subject to palm reversals of this type.

Why should palm orientation errors increase over time? For this question, too, there appears to be a clear answer: palm orientation errors surface most often in fingerspelling, and fingerspelling increases with age, as children become more literate and incorporate more English words into their vocabulary [30]. Indeed, fingerspelling accounted for 110 of the 112 (98.2%) palm orientation errors produced by this child at 14;11. Fingerspelled letters require the pronation of the forearm such that the signer's palm faces outward on all letters except for G and H (produced with supination such that the palm faces inward) and P and Q (produced with pronation and wrist flexion such that the palm faces downward). The tendency to reverse palm orientation on fingerspelled letters was previously

observed for a different child at age 7;5 [1], who produced 61 palm orientation errors, 50 of which were fingerspelled letters produced with inward palm orientation rather than outward. The other 11 errors were midline errors, confirming the patterns observed here: reversals and midline substitutions on fingerspelled letters. Similar to the case discussed here, this child produced a low rate of errors on the other sign parameters (6 movement errors, 1 handshape error, and 0 location errors out of 94 sign tokens), suggesting overall good motor control.

It is important to note that fingerspelling is typically directed toward an interlocutor. In this sense, palm orientation in fingerspelling could also reflect pragmatic competence: the signer must understand that their signing should be produced facing in the direction of their interlocutor. Typically-developing signing children do not produce reversal errors of this type on fingerspelled words; in our previous work no such errors were produced by a sample of 12 deaf children of deaf parents between age 3;7 and 6;9 [1], and to our knowledge there are no other instances of such errors in the literature. The idea that difficulties with pragmatics could underlie the palm reversals documented in the signing of children with ASD suggests a parallel between these errors and the pronoun reversals documented in the speech of hearing children with ASD, as the latter errors have been interpreted as evidence of challenges with understanding how discourse roles shift between interlocutors during conversation [6,54,55].

Despite the frequency with which palm orientation reversal errors occurred on fingerspelled letters, palm orientation reversals also occur somewhat infrequently on other types of signs, such as lexical signs. In our previous work [1], we noted the reversal of palm orientation from inward to outward on the lexical sign FLASHING-LIGHT, and outward to inward on the sign BYE-BYE, both produced by the child described here. In that work as well as in this study, we also find evidence of reversed palm orientation on number signs (which in ASL are formationally similar to fingerspelling), though these errors should be interpreted with caution since there is variability in the production of these signs depending on whether numbers are ordinal, cardinal, or part of a series such as a postal code [56].

One puzzling result is that the palm orientation value for individual signs was variable and unstable. That is, the same sign—especially the same fingerspelled letter—was produced with up to three different palm orientations, and this variability occurred both within the same session as well as across different sessions. If the child had a fixed mental representation of the palm orientation parameter for a given sign, then we would expect him to produce the sign with the same palm orientation value every time he produced that sign. We had hypothesized that differences in imitation style (such as the visual matching strategy, in which the child reproduces signs exactly as they appear from his perspective) could result in mental representations with incorrect palm orientation values [14]. Instead of a fixed but erroneous representation of palm orientation, we propose that the variability of input results in an *unstable* or *underspecified* mental representation of the palm orientation parameter. Children exposed to signs observe signs produced from various angles: whether facing the adult signer head-on, or from the side, or from behind a parent as that adult signs to others, or from every conceivable angle in between. This variability in sign input could result in an unstable value for the palm orientation parameter in the child's mental representation of the sign, or indeed no palm orientation value at all. As it happens, the palm orientation parameter may carry a low functional load compared to the other parameters that signs are composed of. Minimal pairs for palm orientation are signs which differ only in their palm orientation value, proving the phonological contrastiveness of the palm orientation parameter; e.g., CHILDREN versus THINGS [57]. Although minimal pairs for palm orientation do exist in ASL, they appear to be rare, especially compared to minimal pairs for location, handshape, and movement.

We must caution that these results may not be reflective of all signers with ASD. Indeed, ASD is characterized by its diversity of presentation, and this is true both in terms of language ability and motor skills. However, it appears clear that differences in imitation lead both hearing and deaf children with ASD to imitate gestures and produce signs in ways that are unlike typical children, and this paper argues that although motor difficulties appear to be an important factor in the production of palm orientation errors, motor impairment alone cannot account for all of the errors observed. We would

not predict or expect, however, that all signing children with ASD would produce palm orientation reversals. Indeed, such reversals may occur within a subset of children whose language or ASD severity fits a specific cognitive profile, though such a profile has not yet been identified. It is worth noting that the child described here has significant intellectual disability. In contrast, the two children described in our previous report who produced palm orientation reversals did not exhibit intellectual disability [1]; both children were in the average range of intelligence but in the below-average range of language ability. It thus appears plausible that palm orientation reversal errors are unrelated to overall intelligence but could be linked to lower language abilities. A related issue is whether the differences observed could manifest in linguistic structures other than the phonological form of the sign (e.g., role-shift requiring the assumption of different perspectives, or various types of path movements entailed in agreement verbs). We have not observed either of these phenomena due to the overall low level of expressive sign language exhibited by this participant, but future research should investigate whether signing children with ASD experience difficulty with other aspects of the linguistic system that are rooted in motoric, imitative, or other cognitive challenges or differences.

Although the quantity of signs produced in the 12-minute segments increased over time (from 6.33 signs/minute to 16.42 signs/min), the differences in procedures at each time point do not permit direct comparisons. In particular, the increased fingerspelling produced at age 14;11 was largely responsible for the increase in total number of signs produced at the later age. Gains were also observed on the only language measure that was administered at two time points, the ASL RST (at ages 10;2 and 14;11), on which the child increased his raw score from 3 (age equivalent < 3 years) to 12 (age equivalent of approximately 4.5 years). Thus the increase in palm orientation errors occurred *despite* evidence of gains in both receptive and expressive language.

As palm orientation reversals have been documented in a variety of contexts (spontaneous signing, elicited signing, and in the imitation of gestures) and in a variety of populations of children with ASD (hearing children, deaf children, non-signers, and signers), we suggest that such reversals be considered a red flag for ASD diagnosis if they occur past the early developmental period. In particular, if diagnostic and screening instruments are adapted for signing children, we believe that it would be important to include items probing whether or not children produce palm reversals, as such errors rarely occur in typical development beyond the first two years of age.

## 5. Conclusions

This study represents the first longitudinal study of a signer with ASD. It demonstrates that palm orientation errors (both 180-degree palm reversals as well as midline-facing errors) can persist beyond childhood and into adolescence. Such errors are notable for both clinical and theoretical reasons: they could serve as a modality-specific marker of ASD, and as such could be incorporated into adapted diagnostic and screening instruments for signing children, and they also provide insight into the mechanisms (both imitative and motoric) that lead to such errors. Future studies are needed to help clarify how frequently such errors occur in the population of signing children with ASD, and whether there is a specific cognitive profile of children who produce them. These studies will be crucial for a better understanding of why some children with ASD produce these unique errors, and what kinds of differences could lead to their production.

**Author Contributions:** Conceptualization, A.S. and R.P.M.; methodology, A.S. and R.P.M.; data coding and reliability, M.I. and K.R.; writing—original draft preparation, A.S.; writing—review and editing, A.S. and R.P.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by a Doctoral Dissertation Research Improvement Grant BCS-0746009 from the National Science Foundation to R.P.M. and A.S., Autism Speaks Predoctoral Fellowship #4721 to A.S., grant 1F32-DC0011219 from the National Institute on Deafness and Other Communication Disorders to A.S., and a Dean's Scholar Award from the College of Arts and Sciences and Miami University to K.R.

**Acknowledgments:** We thank the research participant and his family for their collaboration over the course of ten years. We also thank Reverb Art + Design for creating the illustrations in Figures 3 and 4.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## Appendix A

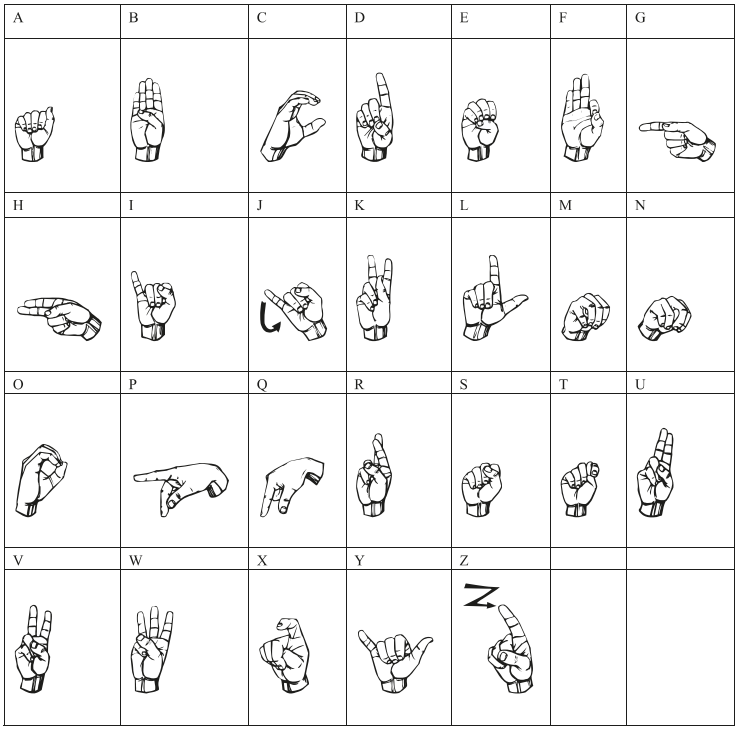


**Figure A1.** ASL Fingerspelling Handshapes.

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# Sensorimotor Research Utilising Immersive Virtual Reality: A Pilot Study with Children and Adults with Autism Spectrum Disorders

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Received: 14 April 2020; Accepted: 26 April 2020; Published: 29 April 2020

**Abstract:** When learning and interacting with the world, people with Autism Spectrum Disorders (ASD) show compromised use of vision and enhanced reliance on body-based information. As this atypical profile is associated with motor and social difficulties, interventions could aim to reduce the potentially isolating reliance on the body and foster the use of visual information. To this end, head-mounted displays (HMDs) have unique features that enable the design of Immersive Virtual Realities (IVR) for manipulating and training sensorimotor processing. The present study assesses feasibility and offers some early insights from a new paradigm for exploring how children and adults with ASD interact with Reality and IVR when vision and proprioception are manipulated. Seven participants (five adults, two children) performed a self-turn task in two environments (Reality and IVR) for each of three sensory conditions (Only Proprioception, Only Vision, Vision + Proprioception) in a purpose-designed testing room and an HMD-simulated environment. The pilot indicates good feasibility of the paradigm. Preliminary data visualisation suggests the importance of considering inter-individual variability. The participants in this study who performed worse with Only Vision and better with Only Proprioception seemed to benefit from the use of IVR. Those who performed better with Only Vision and worse with Only Proprioception seemed to benefit from Reality. Therefore, we invite researchers and clinicians to consider that IVR may facilitate or impair individuals depending on their profiles.

**Keywords:** autism spectrum disorder; ASD; vision; proprioception; self-motion; immersive virtual reality; IVR; HMD; technology

## 1. Introduction

Children with Autism Spectrum Disorders (ASD) can present various types of sensory atypicalities including hypersensitivity, hyposensitivity, and unique patterns of response to sensory stimuli [1], higher reliance on unimodal processing [2], and an extended (hence less precise and specialised) multisensory temporal binding window [3]. These are early symptoms that can be associated with a broad range of cascading delays and impairments [4]. Early motor development might also be affected, as it has been hypothesised that the acquisition of body knowledge develops based on our sensitivity to sensorimotor contingencies (action–consequences correspondence) and multisensory contingencies (correspondence between events in different sensory modalities) [5]. When learning a new movement, there is evidence that children with ASD are less influenced by visual feedback [6] and

that they perform better than neurotypical children when the motor learning is driven by proprioceptive input [7]. For instance, the authors asked typically developing children and children with ASD to reach a target by holding a robotic arm. In some random trials, the robotic arm was perturbed and unexpectedly influenced the children's reaching movement. In the following trial, a learning-from-error effect would lead to an altered movement, which was planned to compensate for the perturbation. The perturbation could be presented to children either through visual feedback (displacement of the cursor representing the robotic arm on the screen) or proprioceptive feedback (a force imposed on the robotic arm). Compared to typically developing children, children with ASD showed a higher sensitivity to when learning from proprioceptive feedback and a lower one when learning from visual feedback [7]. Indeed, motor learning occurs thanks to internal models of action: the association between self-generated motor commands (efferent systems) and sensory feedback from the body and the external world (afferent systems), so that it is possible to predict what would happen as the consequence of an action [6]. Information from muscle, joint, and skin receptors constitute our *proprioception*, the awareness of the position and movement of our body in space which is crucial to the production of coordinated movements [8]. Children with ASD show "an abnormal bias towards reliance on proprioceptive feedback from their own bodies, as opposed to visual feedback from the external world", which might predict impairments in motor control, social skills, and imitation ability [9] (p. 10). In learning motor sequences, adults with ASD also show deficits in the use of vision, which is the sense that neurotypical adults rely on, but preserved proprioception-driven learning [10]. Neurotypical adults have been found to experience a postural illusion (which manifests as a forward lean) when exposed to an intermittent vibratory stimulation of the posterior side of the neck, as long as vision was occluded. On the other hand, those with ASD experienced the illusion even when vision was available, demonstrating limited contribution of vision in modulating proprioception [11]. While the majority of research supports this over-reliance on proprioception, some research has contrastingly related motor impairments in ASD to an over-reliance on vision and proprioceptive deficits [12,13]. However, these studies utilised small sample sizes and limited data analyses. Meanwhile, neuroimaging research has shown associations between ASD severity and asynchronous functional connectivity between visual and motor networks in children at rest [14], reduced functional connectivity between visual areas and somatosensory motor networks, and increased connectivity between the cerebellum and sensorimotor areas in both children and adults at rest [15]. The remaining question is whether there is a general trend of over-reliance on proprioceptive over visual cues at the root of sensorimotor atypicalities in ASD. If that were the case, early interventions could potentially be aimed at increasing the reliance on vision in children with ASD, moving them away from this proprioceptively dominant processing. Such training should improve their sensorimotor functioning, potentially leading to benefits for cognitive, social, and communicative skills.

Immersive Virtual Reality (IVR) is particularly appropriate to this end as it allows for controllable input stimuli and the tracking and monitoring of individuals' actions in a safe learning situation where an individualisation of assessment and training is possible [16]. Moreover, this technology makes it possible to manipulate individual sources of sensory information (e.g., visual, vestibular, or proprioceptive) that are physiologically bound together and induce a mismatch between them to study the role of each sensory modality with respect to accuracy in different tasks [17]. For instance, we can disentangle the contribution of visual and proprioceptive inputs to body perception and movement. In this respect, the most promising IVR tools are head-mounted displays (HMDs), which block out the external world, fully immerse the user in the virtual stimulation, and foster a subjective sense of presence in the virtual world [18]. The result is physiological, emotional, and behavioural responses that are consistent with the physical existence of the virtual world [18]. Despite the broad research and intervention potential offered by HMDs, they have unique features that lead to sensorimotor interactions that do not constitute an exact corollary for real-world experience. Valori and colleagues [19] found that self-motion performance worsened in IVR conditions with vision available relative to the same conditions in

reality and indeed, the way that HMDs deliver visual information has essentially unknown effects on movement and its perception [20].

Most notably, the extant literature seems to neglect a developmental point of view, which is only recently being addressed [21]. It seems that technology-driven peculiarities of IVR and HMDs may induce different sensorimotor effects depending on the user's developmental stage, as has been found in research with neurotypical children and adults. Indeed, when neurotypical people have to learn a walking path while wearing an HMD, adults seem not to benefit from multisensory (visual + self-motion) versus unimodal information, while children of 10−11 years old could benefit from the multisensory learning condition [22]. Therefore, we should investigate the interaction between developmental trajectories of users and the peculiarities of technologies. This would make it possible to understand the unique potentialities and limitations that IVR might have for specific populations with typical or atypical development. At the very beginning of the investigation of the potentialities and limitations related to the use of virtual reality tools for individuals with atypical developmental trajectories and sensory, motor, and cognitive atypicalities, 2D non-immersive systems were preferred due to the technological limits of IVR (graphic quality, limited field of view, temporal lag, size and weight, movement restriction, aftereffects of motion sickness, costs, and accessibility) [23]. Although almost two decades have passed, IVR has greatly improved, and HMDs are sometimes used in research and practice with neurodevelopmental disorders; to our knowledge, only one study has investigated the specific aspects of the interaction between atypical development and the atypicality of interacting with virtual environments. Simões et al. suggest that individuals with ASD may show similar social behaviours (i.e., interpersonal distance) in virtual and real environments, even though neurotypical controls differently interact with a real versus virtual person [24]. We hypothesise that HMDs have unique features that are relevant for people with ASD. This technology seems to intrinsically generate a conflict between vision and proprioception and disrupt the reliability of proprioception [19], potentially reducing its hyper-reliance in ASD. Furthermore, HMDs provide visual information that does not perfectly resemble that of the real world, and they might foster the use of the ventral visual pathway (for object qualities) rather than the dorsal pathway (for movement and spatial aspects of stimuli) [25]. This could suit the visual atypicalities of ASD, which are suggested to present impairments in the dorsal pathway [26], allowing individuals with ASD to interact with the world through the visual mechanisms that are most effective for them. However, several issues should be considered when designing virtual environments for specific purposes in sensorimotor research and interventions for individuals with ASD. Firstly, given that there are usually no binocular cues in IVR, action and perception of depth and motion will be achieved through the ventral stream, which will require much heavier input from the ventral stream than in our daily life [25]. Secondly, more research is needed regarding the role of the dorsal stream in the specific sensorimotor deficits in ASD that would be targeted by an IVR paradigm in order to provide the best possible support for the improvement of sensorimotor skills. Indeed, one of the main goals in the field of IVR technologies is to achieve near-real-life binocular motion and depth perception [27,28].

Although IVR applications for people with ASD are growing for educational, entertainment, and treatment purposes, there is a lack of knowledge about how ASD sensorimotor atypicalities and individual variability might lead to different interactive processes and outcomes. Therefore, the present study presents a method that aims at shedding initial light on the differences between moving and perceiving in reality versus IVR for children and adults with ASD. The knowledge gained through this research will be fundamentally important in informing researchers and clinicians who are using this technology with this specific population.

ASD presents a challenge for any individual involved in understanding, assessing, investigating, and treating those with the disorder. The wide variability of patient profiles requires us as researchers to struggle with methodology, embrace the uncertainty of complex phenomena, and be open, thoughtful, and modest in our research practice [29]. Given the contradictory evidence in the extant literature and the innovative aim of the present research, we adopted an exploratory, descriptive approach. As some

statisticians have recently pointed out, "rather than focusing our study reports on uncertain conclusions, we should thus focus on describing accurately how the study was conducted, what problems occurred, what data were obtained" [30] (p. 262). Therefore, the aim of this pilot is to test the feasibility of the experimental procedure with children and adults with high- and low-functioning ASD, as well as to describe data characteristics. We will highlight the importance of exploring inter- and intra-individual differences, which contain meaningful information for assessment and intervention purposes.

In sum, the aim of the present study is to investigate the extent to which the reliability of visual and proprioceptive information aids the self-motion accuracy of children and adults with ASD. To this end, we utilised a self-turn task and manipulated the way visuo-proprioceptive information was provided among unimodal and multimodal conditions. We also aim to explore whether HMD-delivered IVR, compared to equivalent real environments, affects self-motion accuracy, and to find whether the paradigm is feasible for use with this population.

## 2. Materials and Methods

*Participants.* For this pilot study, we recruited 4 male children (8–13 years old; M = 8.7; SD = 1.2) and 5 male adults (23–39 years old; M = 28.8; SD = 8.3) with a diagnosis of ASD confirmed by their clinicians (see Table 1 for demographic information). The experiment was explained to all parties and informed consent was obtained from parents and professionals responsible for each participant. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of psychology research, University of Padova (Identification code 5A539475A80B5D451B7BC863210C8A61).

**Table 1.** Participants' demographic information.

| Participant | Age | Diagnosis |
|---|---|---|
| C1 | 8 | ASD, ADHD [1], ODD [2], Dysgraphia |
| C2 | 8 | ASD, Mild ID [3] |
| C3 | 10 | ASD, Mild ID |
| C4 | 13 | ASD, Moderate ID |
| A1 | 36 | ASD, Severe ID |
| A2 | 26 | ASD, Mild ID |
| A3 | 20 | ASD, Mild ID |
| A4 | 23 | ASD, Mild ID |
| A5 | 39 | ASD, Severe ID |

[1] ADHD (Attention Deficit Hyperactivity Disorder); [2] ODD (Oppositional Defiant Disorder); [3] ID (Intellectual Disability).

*Setup.* Materials and methods have been described in detail in our previous study with neurotypical children and adults [19]. The employed materials included a soundproof, 2x3 metre testing room with black interior walls where small white clouds were randomly fixed (see Figure 1), illumination, audio communication, and videotaping systems, and the HMD Oculus Gear VR 2016 (101° FOV, 345 g weight, 60 Hz refresh rate) interfaced with a Samsung Galaxy S7 (152 g weight) providing IVR simulations (360° pictures) of the testing room.

*Procedure.* Participants were asked to sit on a swivel chair fixed in the centre of the testing room. For each trial, the experimenter manually rotated the chair a certain degree (passive rotation) from a *start position* to an *end position*. After each passive rotation, participants had to rotate back to the start position (active rotation). Participants' stop position was recorded as the *return position*. The self-turn error was calculated in terms of degrees of absolute difference between the *start position* and the *return position*. Therefore, lower levels of error indicate higher accuracy.

Start, end, and return position data were manually coded by two independent raters of the video recordings. Inter-rater reliability was assessed via intra-class correlation (ICC). The intra-class correlation index (ICC) estimates an ICC = 1, with a 95% confidence interval being $1 < ICC < 1$.

This nearly perfect inter-coder agreement derives from the small mean difference between the two coders' values within the huge range of possible values (0–360). The mean difference between coder A and coder B is minimal ($M_{A\text{-}B} = 0.5$).

**Figure 1.** The testing room.

*Experimental design and conditions.* In a within-subjects multifactorial (2 × 3) design, all participants were randomly exposed to two trials for each of six conditions (a small number of trials was used to keep the experiment as short as possible for participant comfort). The self-turn task was performed in two Environment conditions (Reality and IVR) for each of three Perception conditions (Only Proprioception, Only Vision, Vision + Proprioception). The IVR conditions involved wearing an HMD that showed 360° pictures of perceptually equivalent versions of the reality (R) conditions. The Only-Proprioception (P) condition removed all visual information (with a darkened room or HMD providing no input). The Only-Vision (V) condition limited the access to proprioceptively informative visual landmarks (hiding the participants' body and the room corners) in order to disrupt proprioception, while providing a proprioceptively uninformative visual texture (a pattern of small bright clouds on the walls). The intention was to disrupt proprioception via an alteration of the visual information available without making changes to the proprioceptive information arising from participants' bodies during the passive and active movements. Indeed, previous research has suggested that after being disorientated by a passive rotation in a real environment, people can still detect the position of global landmarks (the room's corners), although they were found to make huge errors in locating surrounding objects [31]. The Vision + Proprioception (VP) condition allowed the participant to access reliable visual and proprioceptive information.

In order to diversify the passive rotations, they were executed both in clockwise and counterclockwise directions, with different amplitudes. Listed below are detailed descriptions of the six experimental conditions.

1. R_P (Reality; only proprioception: no visual information available; the room was completely darkened with no light source available).
2. R_V (Reality; only vision: proprioceptively uninformative visual texture of small bright clouds on the walls. No first-person view of the body or room corners in order to disrupt proprioception by manipulating vision).
3. R_VP (Reality; proprioceptively informative visual cues available, including first-person view of the body and room corners. The visual texture of clouds on the walls is available).
4. IVR_P (HMD on; only proprioception: no visual information available; HMD was worn with no visual input).

5. IVR_V (HMD on; only vision: proprioceptively uninformative visual texture of small bright clouds on the walls. No first-person view of the body or room corners in order to disrupt proprioception by manipulating vision).
6. IVR_VP (HMD on; proprioceptively informative visual cues available, including visible room corners, although the first-person view of the body is not visible. The visual texture of clouds on the walls is available).

All the analyses and graphical visualisations were conducted using the software R (version 3.6.1). The data were described through descriptive statistics and graphical representations, and results were interpreted from an exploratory perspective.

## 3. Results

The first aim of this pilot is to evaluate the feasibility of the experimental procedure with children and adults, even where severe conditions are present. One of the children ("C3", 10 years old) enjoyed the swivel chair and played with it, rotating himself without complying with any verbal instruction provided. Another child ("C4", 13 years old) disliked the testing room and refused to enter it to become familiar with the environment. Data from those participants could not be collected, and the descriptive analyses therefore include seven participants.

The seven participants included here demonstrated that they understood the instructions and task after a short training period. All participants readily wore the HMD. Among them, the two children required several breaks and verbal praise for remaining focused on the task. One of them ("C1") was initially scared by the closing of the room door and by conditions performed in darkness, although he did decide to continue with the experiment. The other ("C2") found the task boring and needed to be continuously motivated. One adult ("A4") performed only the R_P condition and then exited the room, stopping the experiment. Due to technical issues, another adult ("A1") performed the R_VP condition twice and did not perform the IVR_VP condition. The final dataset consisted of 24 observations from children and 50 observations from adults.

The mean self-turn error in the children's sample was 28.4 degrees (SD = 32.3), while in the adults' sample, it was 34.3 degrees (SD = 35.6). The distributions of the observed values have positive skewness, as visualised in Figure 2a,b.

(**a**)

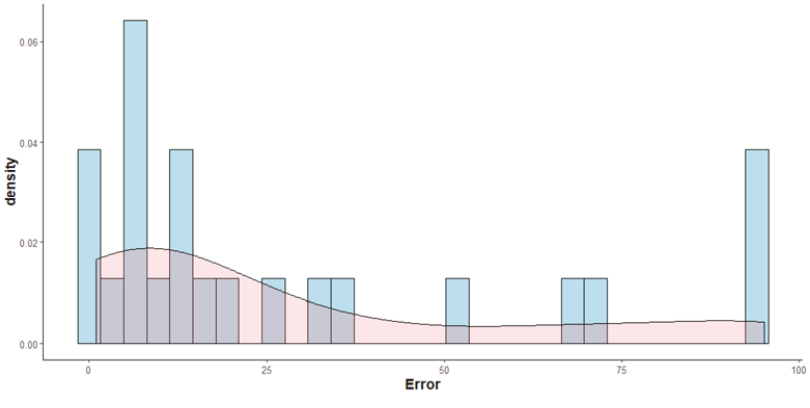


**Figure 2.** *Cont.*

(**b**)

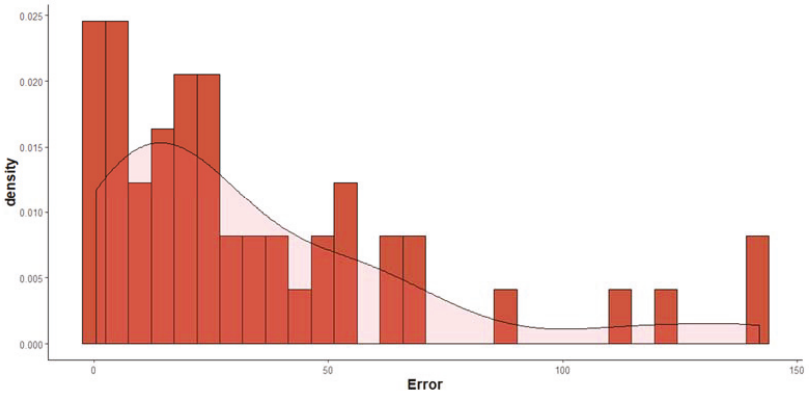


**Figure 2.** (**a**) Distributions of the observed self-turn error. Children ($n_{participants}$ = 2; $n_{observations}$ = 24). (**b**) Distributions of the observed self-turn error. Adults ($n_{participants}$ = 5; $n_{observations}$ = 50).

Exploring the main effect of experimental conditions, it is informative to look at individual observations, where we can appreciate that there is heterogeneity of performance (Figure 3).

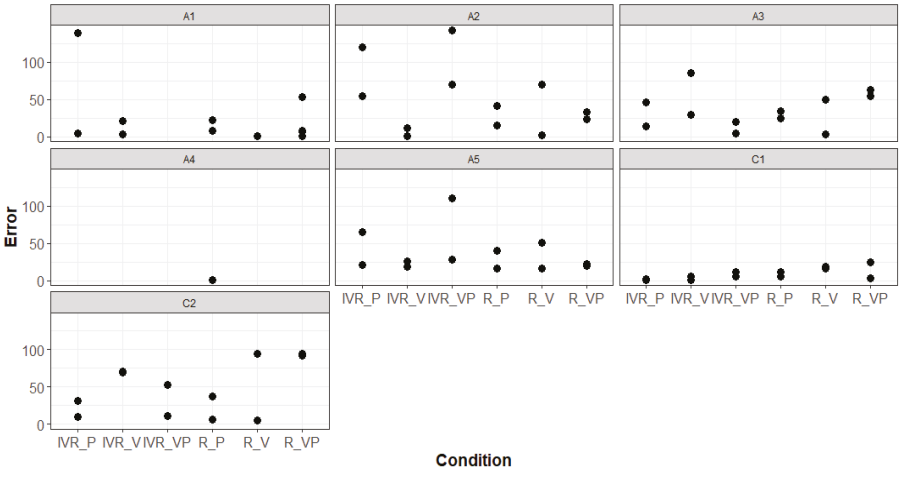


**Figure 3.** Self-turn error of single observations collected by each participant among conditions ($n_{participants}$ = 7; $n_{observations}$ = 74).

Means and standard deviations of self-turn error according to age group and the experimental condition are reported in Table 2.

**Table 2.** Means and standard deviations of self-turn error according to age group and the experimental condition.

| Age Group | Condition | | | | | |
|---|---|---|---|---|---|---|
| | **R_P** | **R_V** | **R_VP** | **IVR_P** | **IVR_V** | **IVR_VP** |
| **Children** | 15.1 (14.8) | 33.6 (40.7) | 53.9 (47) | 10.8 (14.1) | 36.6 (38.9) | 20.4 (22) |
| **Adults** | 20.2 (14.9) | 24.3 (28.2) | 28.4 (21.9) | 58.1 (49.2) | 24.4 (26.9) | 62.5 (55) |

Note: Standard deviations are reported in brackets. ($n_{participants}$ = 7; $n_{observations}$ = 74).

Looking at the marginal role of perception and environment factors, we notice that those participants who perform worse in Only-Vision conditions and better in Only-Proprioception conditions seem to benefit from IVR ("A3"; "C1"; "C2"). Those who perform better with Only-Vision and worse with Only-Proprioception seem to be facilitated in Reality ("A1"; "A2"; "A5") (Figure 4a,b).

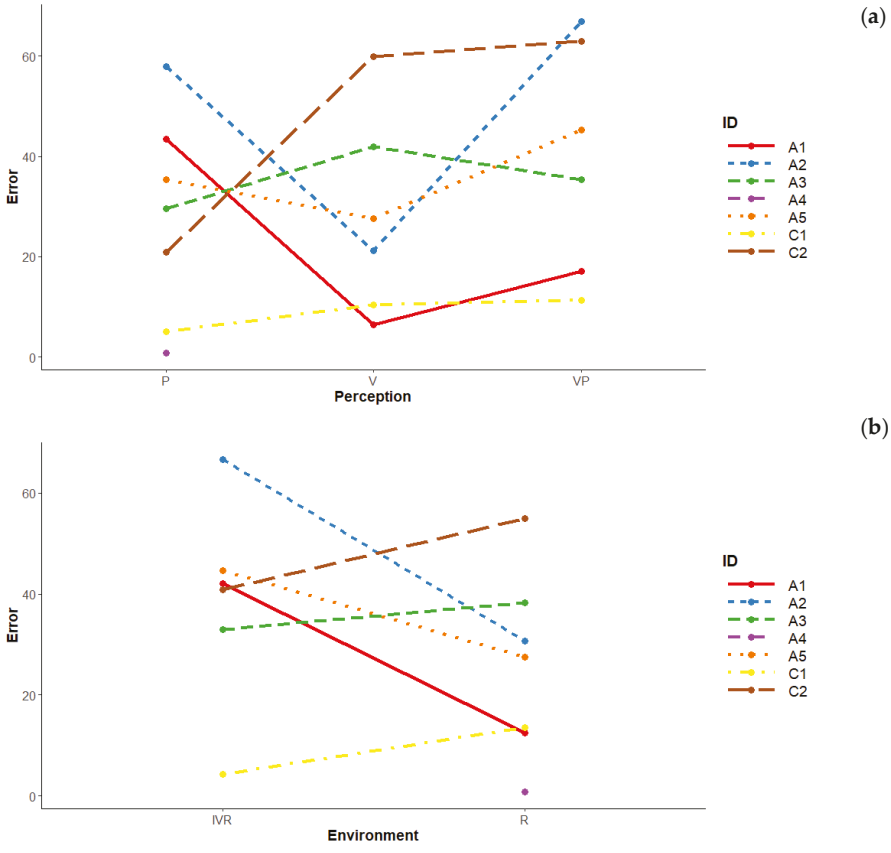


**Figure 4.** (**a**) Mean error made by each participant according to perception (marginalised over the other variables). (**b**) Mean error made by each participant according to environment (marginalised over the other variables).

Trials were equally distributed among the two possible directions (N = 37 trials in clockwise and counterclockwise directions), which do not appear to affect the self-turn error ($M_{clockwise}$ = 32.5; $SD_{clockwise}$ = 34.3; $M_{counterclockwise}$ 32.3; $SD_{counterclockwise}$ = 35.1). The amplitude of passive rotations ranges from 67.5 to 205 degrees (M = 137.2; SD = 38.5). Although the effects of amplitude are not of main interest for this study, consistently with our previous findings [19], this variable is positively correlated with self-turn error. This association seems to be qualitatively different among conditions and age groups (Figure 5a,b). Increasing amplitude appeared to reduce children's accuracy to the greatest extent in Only-Vision conditions performed in both Reality and IVR, while it reduced adults' accuracy to the greatest extent in the Vision + Proprioception condition performed in IVR. Further investigation could specifically address this topic.

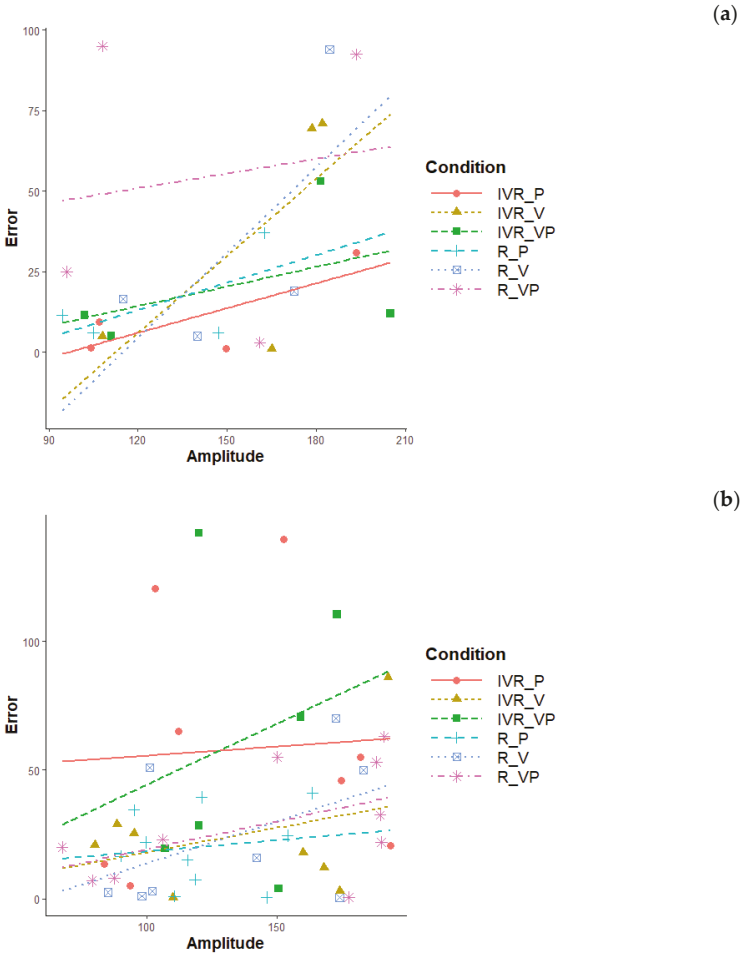


**Figure 5.** (**a**) Regression lines of self-turn error according to rotation amplitude in each condition. Children ($n_{participants}$ = 2; $n_{observations}$ = 24). (**b**) Regression lines of self-turn error according to rotation amplitude in each condition. Adults ($n_{participants}$ = 5; $n_{observations}$ = 50).

## 4. Discussion

This pilot study offers important initial insights regarding IVR research into the use of vision and proprioception in adults and children with ASD. The first finding with respect to feasibility is that all participants, including lower-functioning ones, readily accepted the use of HMD. Therefore, this appears to be a promising tool for research and treatment purposes in the field of severe ASD conditions, which are commonly understudied [32,33]. However, our experimental procedure requires participants to face some obstacles even when they understand the task and perform at a high level of accuracy. In this pilot study, we found that performance tended to fluctuate between within-condition trials and as such, averaging scores would make it difficult to detect an individual's best performance due to interfering factors such as emotional state, motivation, skills of behavioural management, and fluctuations in attention. Future research could adapt the experiment to build a more engaging, game-like activity and include frequent rewards for participation to create a more attractive testing environment for participants. Moreover, a detailed evaluation of within-participant

outlying performances could be run to detect the best performance the individual can show, rather than an average, which obscures these nuances.

As we only present preliminary data from a small sample, we make no inferential claims here. However, we do find this data informative for modest and cautious considerations. First of all, this methodology could show individual differences in the sensory conditions that facilitate self-motion. Moreover, we could distinguish between the individuals that may benefit more or be more impaired by using HMDs. Within the present sample, those who were facilitated by moving when proprioception was available and no vision was present also benefited from IVR. We cannot generalise this result to the whole population of individuals with ASD, but we strongly suggest that researchers and clinicians keep in mind that this technology can either facilitate or impair individuals depending on their profiles. For instance, an IVR training could be particularly effective for individuals who have reduced reliance on vision in reality. We can speculate that the limited use of external stimuli to calibrate internal body-based information might lead to early motor impairments and therefore stereotypy, which refers to restricted repetitive behaviours and interests which reduce the individuals' learning opportunities and interfere with development [34]. Therefore, future research on the potential of IVR training could select people with reduced use of vision for paradigms aimed at learning within IVR and assess outcomes such as improvements in sensorimotor functions, reduction of stereotypies, and cascading benefits on higher-order cognitive and socio-communicative abilities.

Finally, the present pilot study has some limitations, which call for future research using this promising paradigm. The first limitation is that the experimenter manually rotated the participant, and as such, although experimenters were trained to keep a similar speed and method of rotating, the rotation velocity was not perfectly consistent across trials and participants, which could potentially have influenced participants' performance. The second main limitation was the small sample size, which we plan to enlarge in future studies. This would allow us to explore the effect of other relevant factors such as age, comorbidities, and level of general functioning on individual variability. To this end, we aim to extend our measurements and assess other symptoms that could be associated with visuo-proprioceptive atypicalities, such as sensory profile, fine and gross motor abilities, severity of stereotypies and repetitive behaviours, and communicative and social skills.

The method presented here has been previously investigated with neurotypical children and adults [19]. Bayesian model comparison analyses suggested that the sensory information available and the type of environment might result in a perception x environment interaction effect. Therefore, the role of visuo-proprioceptive information might be different in the two environments. Future studies with individuals with ASD could investigate this interaction effect to explore whether different sensory strategies facilitate self-motion in either reality or IVR. Moreover, in a paper in preparation [35], we have further investigated the memory effect of the rotation amplitude (namely, the amount of information to be encoded and reproduced) of our self-turn paradigm, with findings suggesting that the encoding of own body location is facilitated when vision and proprioception are optimally integrated. Consistent with those findings, the present pilot indicates that rotation amplitude might differently affect accuracy across conditions. Our future research with people with ASD could expand on which experimental conditions are most disrupted by memory load.

There is a long way to go, and the present study is just a first indication. As of March 2020, when searching for "Vision" AND "Proprioception" AND "Autism", Scopus provides only 25 documents. Following the first experimental study published in 1983 [36], there was a gap until 2005 for the next theoretical one [37]. Further experimental research is needed to shed light on this early domain-general sensorimotor mechanism that potentially has huge implications for development.

## 5. Conclusions

The present pilot study offers preliminary insights into how the self-motion accuracy of children and adults with ASD is affected by individual differences in the way they rely on vision and proprioception, and in how they interact with real environments and IVR. Preliminary results

suggest that inter-individual variability in sensorimotor functioning has a meaningful impact on the possibility for people with the heterogeneous conditions of ASD to be facilitated by perceiving, moving, and therefore learning in IVR. Importantly, this research also found this paradigm and the use of an HMD to be acceptable and feasible with the present sample, indicating good potential for future research utilising these methods.

**Author Contributions:** Conceptualisation, I.V., P.E.M.-P., R.B. and T.F.; methodology, I.V., P.E.M.-P., R.B. and T.F.; validation, I.V., P.E.M.-P., R.B. and T.F.; formal analysis, I.V.; investigation, I.V., P.E.M.-P., and T.F.; resources, T.F.; data curation, I.V.; writing—original draft preparation, I.V., P.E.M.-P., R.B.; writing—review and editing, I.V., P.E.M.-P., R.B. and T.F.; visualisation, I.V.; supervision, T.F.; project administration, I.V., T.F.; funding acquisition, T.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Beneficentia Stiftung Foundation.

**Acknowledgments:** Many thanks to Marco Godeas and Carlo Marzaroli for their amazing technical support. Our gratitude to Associazione Pro Musica—Public music school and Comune di Ruda (Udine, Italy) for hosting our laboratory and supporting our activities in the last few years. Thanks to F.lli Budai S.r.l. for building the experimental room: a generous gift for which we are very grateful. Last but not least, our projects on ASD greatly benefited from the contributions of brilliant students: Elena Cassano, Miriam Cerasole, Gloria Chiera, Andrea Marchina; Giulia Monsignore. Gigantic thanks to the participants and their families, for trusting us.

**Conflicts of Interest:** The authors declare no conflict of interest.

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**Data Availability:** All data files are available from the OSF public repository at the following URL (https://osf.io/dyf2t/?view_only=746a9829df784d4f9be1312f4e0aa716).

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# P-cresol Alters Brain Dopamine Metabolism and Exacerbates Autism-Like Behaviors in the BTBR Mouse

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Received: 12 March 2020; Accepted: 9 April 2020; Published: 13 April 2020

**Abstract:** *Background:* Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social interaction/communication, stereotypic behaviors, restricted interests, and abnormal sensory-processing. Several studies have reported significantly elevated urinary and foecal levels of *p*-cresol in ASD children, an aromatic compound either of environmental origin or produced by specific gut bacterial strains. *Methods:* Since *p*-cresol is a known uremic toxin, able to negatively affect multiple brain functions, the present study was undertaken to assess the effects of a single acute injection of low- or high-dose (1 or 10 mg/kg i.v. respectively) of *p*-cresol in behavioral and neurochemical phenotypes of BTBR mice, a reliable animal model of human ASD. *Results:* P-cresol significantly increased anxiety-like behaviors and hyperactivity in the open field, in addition to producing stereotypic behaviors and loss of social preference in BTBR mice. Tissue levels of monoaminergic neurotransmitters and their metabolites unveiled significantly activated dopamine turnover in amygdala as well as in dorsal and ventral striatum after *p*-cresol administration; no effect was recorded in medial-prefrontal cortex and hippocampus. *Conclusion:* Our study supports a gene x environment interaction model, whereby *p*-cresol, acting upon a susceptible genetic background, can acutely induce autism-like behaviors and produce abnormal dopamine metabolism in the reward circuitry.

**Keywords:** autism spectrum disorder (ASD); biomarker; *p*-cresol; mouse social behavior; dopamine

## 1. Background

Autism Spectrum Disorder (ASD) is a neuropsychiatric disorder that begins early in childhood and is characterized by deficits in social interaction and communication, repetitive behaviors, restricted interests, and abnormal sensory processing [1]. The incidence of ASD has dramatically risen during the last few decades, reaching the rate of 1 affected in 58 children [2], making autism one of the most widespread disorders in child neuropsychiatry [3,4]. Both genetic and environmental factors contribute to the pathogenesis of ASD [5,6]. A wide variety of environmental factors have been hypothesized to contribute to ASD pathogenesis, but conclusive evidence has been reached for a small minority,

including prenatal infections, some medications (valproic acid, thalidomide, misoprostol, selective serotonin reuptake inhibitors), pesticides, and air pollutants, among others [7].

The complexity of ASD has spurred interest into patient subgrouping strategies, either based on endophenotyping or on biomarkers. Endophenotypes represent familial, heritable and quantitative traits associated with a complex disorder [8,9]. Biomarkers are associated with the disease without necessarily displaying heritability and familiarity; rather, they merely tag for the presence/absence of the disease due to environmental or pathophysiological links, not necessarily of a genetic nature [9]. A reliable set of autism biomarkers could foster earlier and more reliable diagnoses, predict developmental trajectories and treatment response, and identify individuals at high-risk, eventually leading to the establishment of preventive health care strategies, contributing to dissect ASD into more discrete clinical entities, and perhaps even revealing unknown causes of autism, at least in some cases [9].

In recent years, targeted and unbiased metabolomic studies have unveiled a set of potential ASD biomarkers, i.e., small urinary molecules significantly elevated in autistic children [10,11]. Among urinary solutes, *p*-cresol was found to be significantly elevated in autistic children compared to sex- and age-matched controls up until age 8, in two independent samples recruited in Italy and France [12,13]. This finding was later replicated measuring foecal *p*-cresol levels [14,15]. Using an unbiased approach, mass spectrometry-based urinary metabolomics detected *p*-cresol among the 20 solutes best able to differentiate small ASD children from matched controls [11]. Interestingly, elevated urinary *p*-cresol levels were significantly associated with chronic constipation in autistic children, pointing toward slow intestinal transit time as one the main factors allowing greater gut absorption of potentially neuroactive compounds, such as *p*-cresol [16]. The identification of *p*-cresol and of its metabolite *p*-cresylsulphate as two well-known neuroactive uremic toxins poses the question whether, aside from representing a potentially valuable biomarker, the consistent elevation of urinary *p*-cresol detected in young autistic children with chronic constipation may contribute to the clinical severity of their ASD [17]. Preliminary data point toward possible correlations between urinary *p*-cresol concentrations and ASD severity measured using the Childhood Autism Rating Scale (CARS) [12]. Multiple mechanisms could account for the negative influences of *p*-cresol on neural function, ranging from membrane depolarization and increased susceptibility to seizures [18], to decreased $Na^+$-$K^+$ ATPase activity [19], to blunted conversion of dopamine (DA) to norepinephrine (NE) due to inhibition of dopamine-β-hydroxylase [20].

The studies summarized above spur interest into testing *p*-cresol for behavioral effects in animals carrying a genetic predisposition toward autism-like behaviors. Despite several difficulties in developing rodent models with autistic features [21,22], to date, environmental, genetic, and lesion murine models reproducing autism-like behaviors have been developed [22–26]. The present study aims to assess the effects of acute *p*-cresol in a well-established inbred murine model of ASD, the BTBR mouse [23,27,28]. A single low dose of *p*-cresol (1 mg/kg) significantly raises anxiety and hyperactivity, two frequent ASD comorbidities, while acute administration of a higher dose (10 mg/kg i.v.) also exacerbates core symptoms of ASD, blunting interest in a conspecific intruder and enhancing stereotypic behaviors. Brain region-specific neurochemical analyses link these behaviors to parallel, dose-dependent increases in DA turnover in the AMY, nucleus accumbens (NAc) and dorsal caudate putamen (CP).

## 2. Methods

### *2.1. Animals*

Every precaution was taken to minimize animal suffering and the number of animals used. For this study, only BTBR T+tf/J male mice were used. Parental strains were obtained from the Jackson Laboratories (Bar Harbor, ME, USA). After weaning at postnatal day (PND) 28, animals were housed 4 per standard breeding cage with food and water ad libitum on a 12:12 h dark:light cycle (lights on

07:00 a.m.–07:00 p.m.). Only male mice were included in the study to avoid possible variability, due to hormonal fluctuations in female mice. Behavioral experiments were carried at PND 60–70 and were performed on the second part of the day (h 01:00 p.m.–06:00 p.m.). Behavioral tests were performed blind to treatment. Mice were habituated to the behavioral testing room for 1 hour before starting the experiment. Tests were conducted in a sound-attenuated room and recorded through a camera (SSCDC378P, Sony, Tokyo, Japan) connected to a computer. Video were analyzed using the EthoVision video tracking software and the Observer XT program (Noldus information technology, Wageningen, The Netherlands) for automatic and manual recording, respectively.

All groups (CNTR, PC1 and PC10) were submitted to the elevated plus maze, open field motor test, object recognition test [29], and three-chamber social interaction test [30,31], in this order. Behavioral testing was performed 15 min after receiving a *p*-cresol/saline injection. Animals were sacrificed by rapid decapitation 100 min after the injection, heads were frozen and brains were removed and prepared for biochemical assay [32,33].

All experiments of this study were approved by the ethics committee of the Italian Ministry of Health and therefore conducted under license/approval ID #: 10/2011-B, according with Italian regulations on the use of animals for research (legislation DL 116/92) and NIH guidelines on animal care.

### 2.2. P-cresol Treatment

P-cresol was purchased from Sigma-Aldrich (St. Louis, MO, USA), dissolved in saline (0.9% NaCl) and the two different doses (1 or 10 mg/kg) were intravenously delivered by tail vein injection through a micro-cannula to reduce the stress of manipulation. Mice were randomly assigned to experimental groups: (a) naïve, (b) saline-treated controls, and (c) animals that received *p*-cresol 1 mg/kg (P-C1) or (d) *p*-cresol 10 mg/kg (P-C10). Since no difference was recorded between naïve and saline-treated animals, they were grouped together and defined as "control group" (CNTR). Behavior was tested 15 min after the injection.

### 2.3. Elevated Plus Maze

Emotional reactivity and anxiety-like behaviors were measured using the Elevated Plus Maze, a gray plexiglass apparatus with two open arms (27 × 5 cm) and two enclosed arms (27 × 5 × 15 cm) extending from a central platform (5 × 5 cm).

Animals were individually tested for 5 min, and the total number of entries in the open and closed arms, the percentage of entries in the open arms [(open entries/open + closed entries) × 100] and percentage of time spent in the open arms [(time in open arms/time in open + closed arms) × 100] were automatically analyzed using the EthoVision software.

### 2.4. Open Field Test

The apparatus consists in a circular open field, 60 cm in diameter and 20 cm in height. Mice were individually introduced in the empty apparatus and left free to explore the arena for 30 min. Videos from each 30-min Open Field Test session were recorded. Distance travelled (cm) and speed (cm/s) were automatically analyzed using the EthoVision software.

### 2.5. Object Recognition Test

The apparatus is the same as for the Open Field Test (Figure 1C). Each mouse was individually submitted to three 6-minute sessions (Open Field, Pre-Test and Test sessions). At the end of each session, the animal was returned to its home cage for 3 min. All sessions were videotaped and analyzed by an experimenter trained to the Noldus Observer XT event coding software.

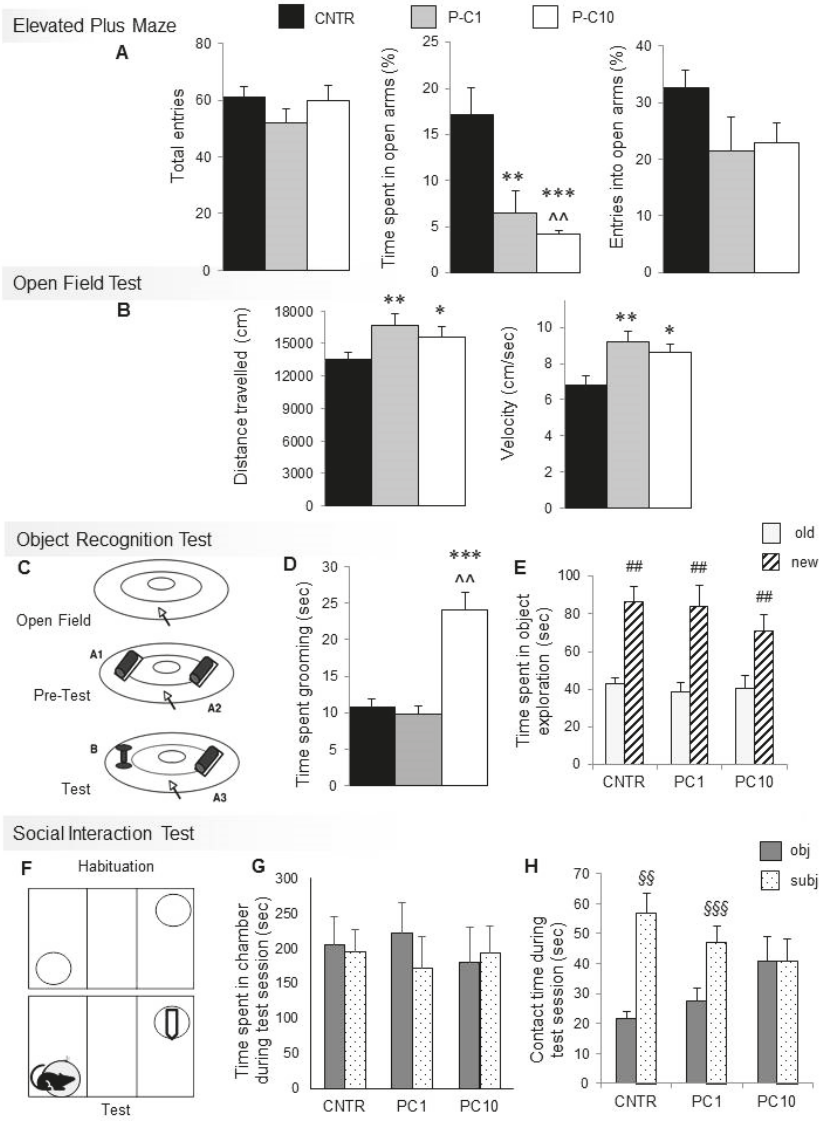


**Figure 1.** P-cresol enhances anxiety-like behaviors, stereotypies, locomotor parameters and hinders social preference in BTBR mice. (**A**) Total entries, % of time spent and entries in open arms in the Elevated Plus Maze. (**B**) Distance travelled and speed in the Open Field Test after acute *p*-cresol treatment. (**C**) Schematic representation of the Object Recognition Test. (**D**) Time spent grooming during the first session of the Object Recognition Test. (**E**) Time spent exploring the novel or familiar object during the test session of the Object Recognition Test. (**F**) Schematic representation of the three-chamber Social Interaction Test. (**G**) Time in object and subject zones during the Social Interaction Test session. (**H**) Time spent in contact with the object or with the social intruder during the Social Interaction Test. Results are shown as mean ± sem. *, **, *** $p < 0.05$, $p < 0.01$, $p < 0.001$ P-C1 or P-C10 vs. CNTR. ^^ $p < 0.01$ P-C10 vs. P-C1, $^{\#\#}$ $p < 0.01$ old vs. new, §§, §§§ $p < 0.01$, $p < 0.001$ subject vs. object.

During the Open Field session, each mouse was left free to explore the arena for 6 min and time spent grooming was measured.

During the pre-Test session, the mouse was introduced in the arena containing two identical objects (A1 and A2: two identical black plastic cylinders of 8 cm in height and 4 cm in diameter, horizontally fixed to a rectangular base), as shown in Figure 1C, and left free to explore. Total time spent exploring two identical objects (A1 and A2) was measured and analyzed.

For the Test session, both objects were substituted, one with object A3, identical to the previous objects, and the other with the new object B (a red and gray plastic spool: 8 cm in height and 5 m in diameter). Object recognition was evaluated by comparing total time spent exploring the novel (B) vs. the familiar (A3) object.

### *2.6. Three-chamber Social Interaction Test*

The apparatus was a three-chamber box made in plexiglass (Figure 1F). Two transparent partitions (23 cm in height) with removable openings divided the box into three identical rectangular chambers (60 cm × 40 cm). The two external chambers contained two perforated plexiglass cylinders, used to enclose stranger BTBR mice. The test consisted in two 10 min sessions, encompassing the Habituation session and the Sociability Test session. Immediately after the Habituation session the animal was confined to the center chamber while an unfamiliar strain-, sex-, and age-matched adult intruder (subject) or an object were placed inside the cylinders. Videos were recorded and analyzed both automatically and manually, using the EthoVision and Observer XT programs. Time spent in each chamber, time spent in contact with the two cylinders, distance travelled and speed were recorded and analyzed.

### *2.7. Biochemical Assay*

Biochemical assays were performed as previously described [32,33]. Briefly, frozen brains were fixed vertically on the freezing microtome pate. Punches were obtained from 300 μm-thick brain slices (coronal sections). Stainless steel tubes of 0.8, 1.0, or 1.5 mm inside diameter were used. Coordinates were measured as follows: medial pFC, two slices from section 80 to section 130 (1.5 mm tube); NAc, three slices from section 151 to section 201 (1.0 mm tube); CP, 4 slices from section 151 to section 230 (1.5 mm tube); AMY, 5 slices from section 251 to section 350 (0.8 and 1.0 mm tube); HIP, 3 slices from section 301 to section 350 (0.8 and 1.0 mm tube; including CA1, CA2 and CA3 fields). Punches were stored in liquid nitrogen until the day of analysis. Frozen tissues were then weighed and homogenized in 0.05 M $HClO_4$. Homogenates were centrifuged at 14,000 rpm for 20 min at 4 °C. Tissue levels of DA, NE, 5-HT and their metabolites were assessed using HPLC. The HPLC system consists of an Alliance (Waters) system and a coulometric detector (ESA Model 5200A Coulochem II) provided with a 5011 high sensitivity analytical cell and a 5021 conditioning cell, the potential being set at 0.450 mV and 0.100 mV, respectively. A Nova-Pack Phenyl column and a Sentry Guard Nova-Pack pre-column were purchased from Waters Assoc. Flow rate was 1 ml/min. The mobile Phase consisted of 3% methanol in 0.1 M Na-phosphate buffer pH 3.0, 0.1 mM, $Na_2EDTA$ and 0.5 mM 1-octane sulphonic acid Na salt.

### *2.8. Statistical Analysis*

Behavioral parameters recorded in the Elevated Plus Maze and Open Field Test were analyzed using one-way ANOVAs to detect group effects (three levels: CNTR, P-C1, P-C10), followed by a post-hoc Duncan's test. For the Object Recognition Test, the total time spent exploring the familiar (A3) vs. the novel (B) object during the test session were analyzed by two-way ANOVA for repeated measures ("group", three levels: CNTR, P-C1, P-C10 as between factor; "object", two levels: A3 and B as within factor). Simple effect analysis of the factor "object" was also performed within each group. Similarly, for the Social Interaction Test time spent in each chamber and time spent in contact with the two cylinders were analyzed by two-way ANOVA for repeated measures ("group" three levels: CNTR, P-C1, P-C10 as between factor; "zone", two levels: object and subject as within factor). Distance

travelled and speed by treatment group were analyzed using one-way ANOVA, followed by Duncan's post-hoc test. Data are presented as mean ± sem.

One-way ANOVAs, followed by a post-hoc Duncan's test, were used for statistical analysis of the effects of treatment (three levels: CNTR, P-C1, P-C10) for each amine and metabolite (ng/g wet weight) within each brain region.

## 3. Results

### 3.1. P-cresol Enhances Anxiety-like Behaviors in BTBR Mice

The Elevated Plus Maze test is based on the natural inclination of mice to avoid open, elevated and bright places, in spite of their tendency to actively explore novel environments. Results are shown in Figure 1A (CNTR, $n = 10$; P-C1, $n = 8$; P-C10, $n = 8$ mice). The percentage of time spent in the open arms by the CNTR group (17.13%) is consistent with previous studies [34]. P-cresol (1 and 10 mg/kg) profoundly decreases the percentage of time spent in the open arms ($F_{2,23} = 10.632$; $p < 0.001$), without significantly affecting the total number of entries ($F_{2,23} = 1.187$; $p = 0.32$) and the percentage of entries in the open arm ($F_{2,23} = 1.644$; $p = 0.21$). Hence, both low and high *p*-cresol doses increase anxiety-like behaviors in BTBR mice tested using the Elevated Plus Maze.

### 3.2. Locomotor Activity is Enhanced by p-cresol in the Open Field Test

Results from the Open Field Test are displayed in Figure 1B (CNTR, $n = 10$; P-C1, $n = 9$; P-C10, $n = 7$). Both low- and high-dose *p*-cresol significantly enhanced distance travelled ($F_{2,23} = 5.826$; $p < 0.01$) and speed ($F_{2,23} = 5.914$; $p < 0.01$) compared to control mice, already yielding hyperactivity at low *p*-cresol doses.

### 3.3. P-cresol Enhances Motor Stereotypies without Modifying Object Recognition and Discrimination Behaviors

During the first Object Recognition Test session (Figure 1C), time spent grooming was measured (CNTR, $n = 8$; P-C1, $n = 7$; P-C10, $n = 7$). Figure 1D shows that the P-C10 group spent significantly more time self-grooming compared with controls and P-C1 animals ($F_{2,19} = 18.12$; $p < 0.001$), who do not differ from each other. A partial dose-dependent shift from hyperactivity to stereotyped behaviors was thus recorded.

Time spent exploring two identical objects during the Pretest session of the Object Recognition Test did not differ between controls and treatment groups (mean ± sem: CNTR = 80.27 ± 6.59; PC-1 = 88.09 ± 6.25; PC-10 = 67.55 ± 11.92; $F_{2,23} = 1.426$ $p = 0.264$, data not shown), demonstrating unchanged interest in object exploration. Similar results were obtained during the Test session (Figure 1E), indicating that *p*-cresol does not significantly influence the ability to discriminate novel vs. familiar objects ($F_{2,19} = 0.897$; $p = 0.424$).

### 3.4. High Dose p-cresol Thwarts Preference for Social Interaction

Behavioral results from the three-chamber Social Interaction Test are displayed in Figure 1G,H (CNTR, $n = 6$; P-C1, $n = 7$; P-C10, $n = 7$). No treatment effect was recorded on general motor activity neither during the habituation session (distance travelled: $F_{2,16} = 3.342$; $p = 0.054$; speed: $F_{2,16} = 1.544$; $p = 0.237$; time spent in each chamber: $F_{2,16} = 0.276$; $p = 0.763$), nor during the Sociability Test session (distance travelled, $F_{2,16} = 1.504$; $p = 0.243$; speed: $F_{2,16} = 1.572$; $p = 0.229$; time spent in each chamber $F_{2,16} = 0.164$; $p = 0.85$) (Figure 1G). Time spent sniffing the cylinders did not differ during habituation ($F_{2,16} = 0.263$; $p = 0.77$), whereas a significant treatment effect was recorded during the Sociability Test over time spent in contact with the cylinders containing subject vs. object ($F_{2,16} = 6.241$; $p < 0.01$). In fact, CNTR and low-dose cresol-treated animals (P-C1) maintained a significant preference for the social stimulus, while high-dose cresol-treated animals (P-C10) lost their social preference, spending

the same amount of time sniffing the two cylinders containing either the conspecific intruder or the object (Figure 1H).

### *3.5. P-cresol Enhances Dopamine Metabolism in NAc, CP and AMY*

Neurochemical data concerning brain levels of monoamines and their metabolites assessed in medial pFC, HIPP, AMY, CP and NAc are summarized in Table 1 and Figure 2 (CNTR, $n = 9$; P-C1, $n = 6$; P-C10, $n = 6$). Significant treatment effects were recorded in NAc, CP and AMY on levels of DA (NAc $F_{3,18} = 21.358$; $p < 0.001$; CP: $F_{3,15} = 13.028$; $p < 0.001$; AMY: $F_{3,15} = 3.267$; $p < 0.05$), HVA (CP: $F_{3,15} = 8.988$; $p < 0.001$; NAc: $F_{3,18} = 6.649$; $p < 0.01$), and DOPAC (NAc: $F_{3,18} = 9.886$; $p < 0.001$; CP: $F_{3,15} = 5.851$; $p < 0.001$; AMY: $F_{3,15} = 3.482$; $p < 0.05$) (Figure 2B). DA turnover was largely enhanced in NAc and CP and only by high-dose *p*-cresol (P-C10); whereas in AMY, both low- and high-dose *p*-cresol were equally effective (Figure 2B). No significant change was recorded for norepinephrine and 5-HIAA, whereas 5-HT levels were increased only in the CP following the higher dose of *p*-cresol ($F_{2,16} = 8.927$; $p < 0.01$) (Table 1). No treatment effect was detected in medial pFC and HIPP for any monoamine or metabolite level (Table 1).

**Table 1.** Neurochemical analysis of monoamine and metabolite levels (ng/g wet weight) assessed in medial prefrontal cortex, hippocampus, amygdala, caudate putamen and nucleus accumbens.

| | | DA | DOPAC | HVA | NE | 5HT | HIAA |
|---|---|---|---|---|---|---|---|
| **pFC** | **CNTR** | 342.95 ± 99.78 | 44.96 ± 9.21 | 43.95 ± 7.91 | 179.12 ± 26.24 | 826.28 ± 116.29 | 266.67 ± 51.36 |
| | **P-C1** | 305.53 ± 57.11 | 43.79 ± 11.19 | 37.07 ± 6.91 | 132.30 ± 25.35 | 607.64 ± 74.63 | 152.62 ± 15.13 |
| | **P-C10** | 423.87 ± 138.8 | 76.9 ± 24.84 | 42.91 ± 14.9 | 139.42 ± 36.39 | 881.03 ± 207.31 | 171.06 ± 39.93 |
| **HIPP** | **CNTR** | 155.18 ± 15.24 | 41.73 ± 15.71 | 29.98 ± 2.79 | n.d. | 641.22 ± 173.86 | 378.98 ± 59.82 |
| | **P-C1** | 113.92 ± 26.27 | 21.54 ± 7.92 | 22.77 ± 3.72 | n.d. | 425.17 ± 92.36 | 327.61 ± 102.74 |
| | **P-C10** | 119.47 ± 40.91 | 37.29 ± 12.97 | 30.65 ± 7.65 | n.d. | 365.6 ± 145.62 | 314.2 ± 99.42 |
| **AMY** | **CNTR** | 858.36 ± 112.78 | 68.98 ± 6.62 | 57.41 ± 11.05 | 274.34 ± 103.69 | 193.46 ± 42.02 | 149.84 ± 55.3 |
| | **P-C1** | 2292.71 ± 526.75 * | 187.95 ± 38.11 * | 122.05 ± 26.41 * | 314.18 ± 89.95 | 356.62 ± 109.27 | 212.38 ± 88.17 |
| | **P-C10** | 2197.45 ± 992.31 * | 179.38 ± 30.12 * | 140.21 ± 23.75 * | 283.15 ± 48.49 | 330.85 ± 59.27 | 226.03 ± 36.56 |
| **CP** | **CNTR** | 5284.18 ± 1015.8 | 584.97 ± 186.65 | 251.81 ± 33.64 | 35.46 ± 8.61 | 159.61 ± 31.77 | 133.99 ± 28.56 |
| | **P-C1** | 5499.14 ± 842.38 | 327.32 ± 56.27 | 240.48 ± 43.35 | 46.41 ± 8.61 | 183.88 ± 31.09 | 130.22 ± 38.39 |
| | **P-C10** | 16270.59 ± 2153.37 ***,### | 1176.26 ± 223.34 ***,### | 658.32 ± 97.93 ***,### | 63.11 ± 12.83 | 341.1 ± 34.82 **,## | 242.73 ± 49.04 |
| **NAc** | **CNTR** | 5623.89 ± 1050.64 | 506.55 ± 91.15 | 412.55 ± 87.32 | 1214.04 ± 390.84 | 1191.69 ± 355.05 | 459.72 ± 126.19 |
| | **P-C1** | 5035.05 ± 1134.2 | 504.3 ± 119.73 | 276.01 ± 49.36 | 1359.63 ± 200.81 | 1232.82 ± 357.02 | 305.25 ± 63.12 |
| | **P-C10** | 16156.11 ± 1812.97 **,## | 1698.82 ± 325.04 **,## | 817.35 ± 91.08 *,## | 1883.69 ± 626.27 | 1360.47 ± 512.3 | 480.62 ± 189.73 |

Data are shown as mean ± sem. Highlighted in bold, significant effects of group x amine or metabolite. CNTR, $n = 9$; P-C1, $n = 6$; P-C10, $n = 6$. *, **, ***$p < 0.05, 0.01, 0.001$ P-C1 or P-C10 vs. CNTR. #, ##, ### $p < 0.05, 0.01, 0.001$ P-C10 vs. P-C1.

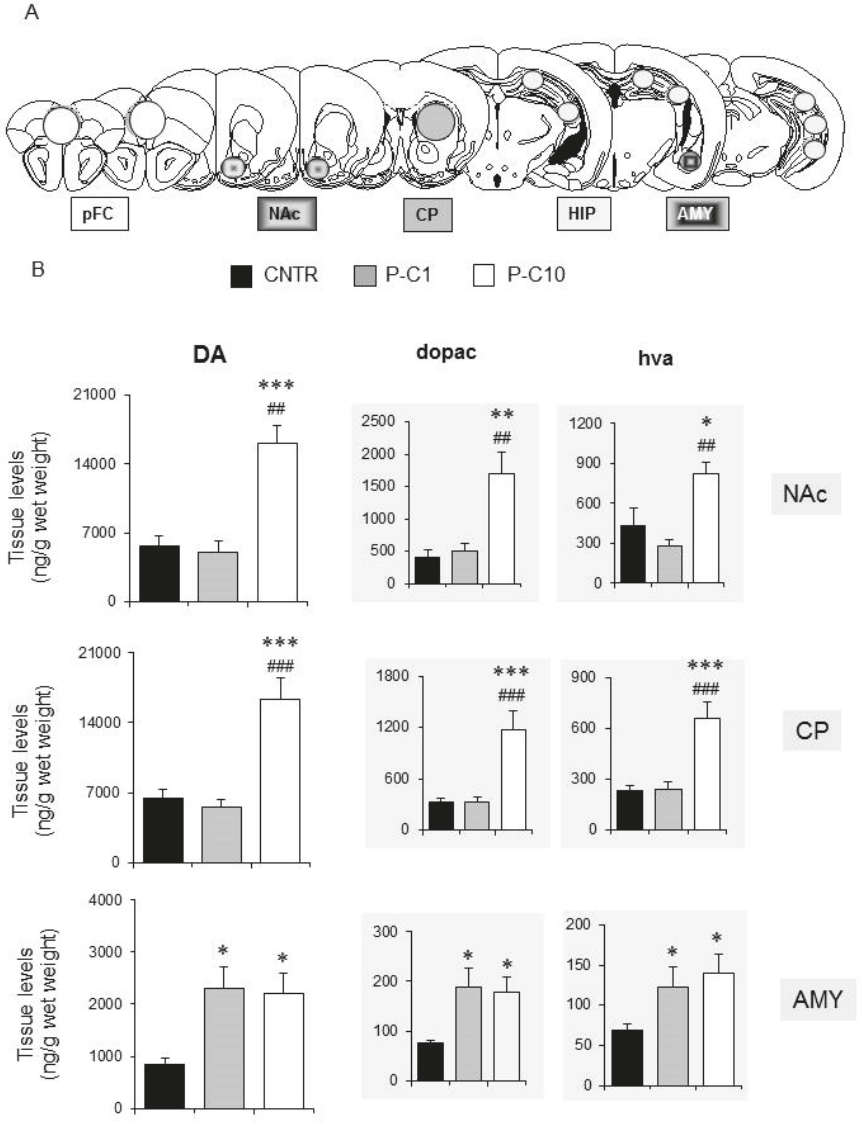


**Figure 2.** P-cresol enhances tissue levels of dopamine and its metabolites in the amygdala, caudate putamen and nucleus accumbens of BTBR mice. (**A**) Tissue levels of DA, DOPAC, HVA, NE, 5-HT and 5-HIAA, measured in medial pFC, NAc, CP, HIP, AMY. (**B**) Tissue levels of DA, DOPAC, HVA, measured in NAc, CP and AMY. CNTR, $n = 9$–10; P-C1, $n = 6$, P-C10 $n = 6$. Data are expressed as mean ± sem ng/g wet weight. *, **, *** $p < 0.05$, 0.01, 0.001 P-C1 or P-C10 vs. CNTR group. ##, ### $p < 0.01$, 0.001 P-C10 vs. P-C1 (treatment effect) by Duncan's post-hoc test following one-way ANOVAs. Abbreviations: AMY: Amygdala; CP: Caudate Putamen; DA: dopamine; DOPAC: 3,4-Dihydroxyphenylacetic acid; HIP: Hippocampus; HVA: Homovanillic acid; NAc: Nucleus Accumbens; pFC: preFrontal Cortex.

## 4. Discussion

In the present study, acute *p*-cresol administration to BTBR mice, a reliable animal model of ASD [23,27,28], elicited autism-like behaviors and enhanced dopaminergic turnover both in the AMY, and in the dorsal and ventral striatum. Importantly, behavioral abnormalities elicited by *p*-cresol in BTBR mice strikingly resemble core symptoms and co-morbid disorders clinically observed in human autistic individuals. On the one hand, excessive interest in objects over social interaction and stereotypic behaviors represent two of the hallmarks of an ASD diagnosis in humans [1]. Additionally, hyperactivity and anxiety are among the most frequent co-morbidities in autistic patients, with ADHD and anxiety disorders being diagnosed in 33%–37% and in 39.6% of ASD cases, respectively [35,36]. BTBR mice are an inbred strain spontaneously displaying autism-like behaviors [23,27,28]. These behavioral abnormalities likely stem from strain-specific genetic underpinnings involving neurodevelopmental genes, like kynurenine 3-hydroxylase (*Kmo*), Disrupted in Schizophrenia (*Disc1*) and exostosin 1 (*Ext1*) [28]. The induction of hyperactivity in the Open Field Test, but not in the 3-chamber Social Interaction Test, most likely represents only an apparent contradiction, because the more interesting social interaction apparatus is able to engage motivated exploratory behaviors in mice that can "cover" the spontaneous hyperactivity visible in the Open Field Test. In addition, differences in session duration between the two tests (30 min in the Open Field Test vs. 10 minutes in the Social Interaction Test) can further influence the expression of hyperactivity in treated BTBR. Instead, a large body of literature reports a lack of sociability in BTBR using the three-chambered social approach, although data showing that BTBR control mice display significant sociability [37–40] or a non-significant preference for subject exploration are also present (see Figure 1B in ref. [40], Figure 3B in ref. [41], and Figure 3B in ref. [42]). One possible explanation for these discrepancies is that genetically-driven ASD-like behaviors in the BTBR strain may spontaneously be under threshold and may emerge to a different extent depending upon experimental manipulations, handling or treatments [37]. Furthermore, discrepancies due to different choice of intruder (conspecific vs. different strain) in the Social Interaction Test cannot be excluded (in present study we used a BTBR conspecific intruder). Baseline control behavioral parameters recorded in our BTBR mice in the Elevated Plus Maze, Object Recognition Test and Social Interaction Test are absolutely in line with previous studies from our lab [29,32,43,44] and are coherent with the overall literature [45–47], although absolute values predictably differ, likely due to differences in housing environment, animal handling, and test settings. Finally, blunted social preference in the three-chamber test could conceivably stem from enhanced anxiety rather than reflecting a real social interaction deficit. While we cannot exclude contributions by anxiety to this behavior, the emotional reaction of BTBR mice to the objects during pre-test and test sessions of the Object Recognition Test did not differ between groups, as all groups spent the same time exploring objects. Most importantly, both low- and high-dose *p*-cresol produced anxiety-like behaviors in the Elevated Plus Maze. Therefore, if anxiety played a pivotal role in reducing social preference, the lower *p*-cresol dose should have also been effective. In summary, our results collectively support a gene x environment interaction model, whereby, acting upon a susceptible genetic background, *p*-cresol triggers anxiety and hyperactivity at a low dose, while boosting core autism-like symptoms at the higher dose.

Behavioral abnormalities are paralleled by neurochemical alterations, mainly involving the dopaminergic turnover. This interpretation is in line with long-standing evidence of dopamine-β-hydroxylase inhibition by *p*-cresol [20] and with the proportionate increase in DA and its metabolites, supporting increased DA accumulation, release and catabolism (both intra- and extra-cellular). However, the measurable, albeit non-significant, increase in NE recorded in several brain regions displaying increased DA and its metabolites (Table 1) indicates that enhanced DA synthesis may also contribute to cresol-induced dopaminergic imbalance. On the one hand, levels of DA and its metabolites were dose-dependently increased in the ventral and dorsal striatum, where only the higher *p*-cresol dose was effective (Figure 2B). On the other hand, dose-independent effects were recorded in the AMY, where low- and high-dose *p*-cresol were equally effective in boosting DA turnover (Figure 2B). This regional distribution and dose-dependency fit well with the pattern

of behavioral abnormalities recorded in these same animals. Low- and high-dose *p*-cresol were equally effective in reducing time spent in the open arms at the Elevated-Plus Maze and in enhancing locomotor activity (Figure 1A,B). Instead, only high-dose *p*-cresol significantly increased stereotypic behaviors and blunted social interaction(Figure 1D,H). This trend resembles the effects of acute amphetamine in rodents, yielding hyperactivity at low doses and stereotypic behaviors (sniffing and grooming) at higher doses [48,49]. Drosophila melanogaster carrying the ASD-associated hDAT ΔN336 variant, which impairs DA uptake while sparing DA efflux, displays behavioral abnormalities that are strikingly overlapping with those recorded here following acute *p*-cresol—namely increased fear, impaired social interactions, and enhanced locomotion [50]. Modest increases in 5-HT levels parallel the much larger changes observed in levels of dopamine and its metabolites (Table 1). We cannot exclude synergistic serotoninergic contributions to cresol-induced behavioral effects, since 5-HT transporter KO mice display at least some autism-like behaviors, including social deficits and increased anxiety [51]. However, changes in brain 5-HT levels are relatively minor compared to changes in DA and never reach statistical significance, except in the striatum following high-dose *p*-cresol (Table 1). Furthermore, changes in 5-HIAA levels are even more modest, and there is only partial overlap between serotoninergic neurochemical parameters and behavioral changes. Collectively, serotoninergic contributions to cresol-induced behavioral abnormalities may seemingly play a secondary role at best. Instead, our data strongly reinforce the "dopamine hypothesis" of ASD [52], pointing toward the existence in autistic brains of two distinct dopaminergic activation thresholds: a lower threshold in the AMY to boost anxiety and hyperactivity, and a higher threshold in ventral and dorsal striatum to produce stereotypic behaviors and to divert motivational drives from interaction with conspecific animals to inanimate objects. D1 receptor activation or D2 receptor knock-out in the dorsal striatum have been shown to yield autistic-like behaviors in mice [53]. In line with this evidence, BTBR mice display blunted DRD2 signaling and responsiveness to extracellular DA in the presence of preserved DRD2 mRNA and protein levels [54]. On the other hand, comparable DRD1 expression and responsiveness to DA was recorded in BTBR and in C57Bl6 mice [54]. Altogether, much of the current literature on the motivational circuitry in ASD underscores reward-processing deficits towards social and monetary incentives [55,56]. Instead, results displayed in Figure 1H promote a more balanced view, whereby reduced DA activation by social stimuli may be seemingly paired with preserved or even enhanced DA activation by exposure to inanimate objects or by sensory self-stimulation [57–59]. Future experiments will have to extend the present findings, identifying the receptor and signaling pathways mediating the dopaminergic effects recorded in our experiments, and to explore whether the activation of DA turnover by *p*-cresol contributes to favoring LTP-based synaptic plasticity in the NAc [60], possibly fostering "addictive" attitudes towards routines, objects, or absorbing interests including internet and videogames.

Urinary and foecal levels of *p*-cresol have been consistently found elevated in autistic children compared to typically developing controls [11–16]. Preliminary evidence suggests that high urinary *p*-cresol may be clinically associated with greater autism severity and history of behavioral regression [12,17]. P-cresol is not produced by human cells, which lack *p*-hydroxyphenylacetate decarboxylase (pHPAD), the final enzyme of tyrosine transformation into *p*-cresol [17]. Hence, urinary *p*-cresol is either absorbed through the skin, the gut and the lungs from a variety of environmental sources (listed in Table 2 in ref. [17]), or it is produced by gut bacterial strains able to express pHPAD. The primary origin of urinary *p*-cresol elevation in autistic children remains to be determined, as does the reason for its normalization after age 8. However, its association with chronic constipation and longer intestinal transit time supports greater *p*-cresol absorption through the gut, while no association with the "leaky gut" was observed [16]. Chronic constipation thus likely represents a broad, non-specific facilitator of neurotoxic effects exerted by environmental and gut-derived compounds.

The present results raise further interest into *p*-cresol, not only as an ASD biomarker but also as a potential contributor to autism pathogenesis, by boosting DA turnover in specific brain regions of autistic individuals. P-cresol is certainly not the only neuroactive exogenous compound produced by

gut bacteria and able to negatively affect behavior. Propionic acid, a short chain fatty acid produced by anaerobic gut bacteria including Clostridia and Propionibacteria, has been shown to produce a variety of behavioral, immune, mitochondrial effects in rodent models closely resembling human ASD [61]. Studies of urinary and foecal levels of propionic acid in autistic children compared to typically developing controls have yielded conflicting results [14,15]. Nonetheless, this compound could indeed play a pathoplastic role in specific patient subgroups, which need to be better defined at the clinical level. Meanwhile, additional tryptophan-derived gut bacterial compounds were found significantly elevated in the urines of autistic children, namely indolyl 3-acetic acid, indoxyl sulfate, and indolyl lactate [11]. These compounds have not yet been thoroughly assessed for possible neuroactive behavioral effects.

## 5. Limitations

The main limitation of the present study is the lack of a reversal experiment, showing that abnormal behaviors are corrected by administering dopamine receptor antagonists. Due to practical constraints, sample sizes of BTBR mice are relatively small, but 4–5 different litters were used for behavioral experiments and behavioral data appear reasonably consistent among different litters. In fact, all significant differences between control vs. cresol-treated animal mean values displayed in Figure 1 are at least three times larger than inter-litter S.E.M.s per each sample, with the sole exception of the Social Interaction Test (object vs. subject contact time) were P-C10 and controls differ 2.47 times the interlitter S.E.M. values of controls. Repetitive behaviors/restricted interests were assessed only by measuring stereotypic motor activity in the open field test, and not by applying specific tests designed to quantify mouse behaviors corresponding more closely to this diagnostic criterion. Locomotor activity data could have provided additional information if broken down into bins of 3–5 min, allowing an assessment of how quickly the mice habituate to the open field, and the time course of *p*-cresol effects. Finally, urinary baseline levels of endogenous *p*-cresol should be measured and compared among different inbred mouse strains because, if particularly elevated in BTBR mice, they could promote their autism-like phenotypic features and contribute to the behavioral abnormalities induced by exogenous *p*-cresol administration. In addition to addressing these limitations, our follow-up study will involve in parallel both the hypersociable C57Bl/6 mice and the ASD model BTBR mice, to further test the hypothesis that the behavioral abnormalities exacerbated by acute *p*-cresol are the result of a BTBR-specific gene x environment interaction.

## 6. Conclusions

This study demonstrates that acute *p*-cresol administration to an animal model of ASD induces behavioral abnormalities closely resembling core symptoms of ASD and comorbidities frequently observed in autistic individuals. These results underscore the importance of gene x environment interaction models, able to merge genetic predisposition and evidence-based environmental exposure to specific neurotoxic compounds into a unitary scenario. From a mechanistic standpoint, these results move the field beyond well-established paradigms in the autism literature, such as the imbalance between glutamate and GABA to explain insistence on sameness and the co-morbidity with epilepsy [62], or the role of 5-HT in reference to hyperserotonemia, disruption of circadian rhythmicity, neuroinflammation and neuronal excitability [63–65]. In a complementary view, they point toward critical dopaminergic roles in autistic symptoms as being relevant as stereotypic behaviors, hyperactivity, anxiety and motivational drive towards inanimate objects. Thirdly, urinary gut-derived neurotoxic compounds, such as *p*-cresol, could serve as useful ASD biomarkers, whose specificity now deserves to be assessed in samples of young non-autistic children affected with chronic constipation. Finally, the correction of chronic constipation and microbiota transfer therapy represent two reasonable and testable approaches, aimed at partly ameliorating autistic behaviors by reducing the absorption of neurotoxic compounds of environmental origin or derived from specific gut-bacterial strains [66]. Studies addressing the efficacy of these therapeutic approaches will largely benefit from parallel

assessments of urinary biomarkers, such as *p*-cresol and other gut-derived compounds, in order to provide mechanistic insights into their effects on the longitudinal time course of autistic symptoms.

**Availability of data and materials:** The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

**Author Contributions:** Conceptualization, T.P., R.S., S.P.-A., and A.M.P.; Data curation, M.C., E.F., A.C., R.V. and L.T.; Formal analysis, E.F., A.C. and R.V.; Funding acquisition, T.P.; Investigation, M.C., A.C. and R.V.; Methodology, M.C., R.S., A.C. and R.V.; Supervision, T.P., S.P.-A. and A.M.P.; Writing—original draft, T.P., E.F. and A.M.P.; Writing—review and editing, T.P., LT. and A.M.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by Ministry for Education, University and Research (Grant n. RBFR10RZ0N_002) to TP and RV and by the Italian Ministry of Health (Grants n. NET-2013-02355263 and CCR-2017-9999901) to AMP.

**Conflicts of Interest:** The authors declare that they have no competing interests.

## Abbreviations

| | |
|---|---|
| AMY | Amygdala |
| ASD | Autism Spectrum Disorder; |
| CARS | Childhood Autism Rating Scale; |
| CNT | Control; |
| CP | Caudate Putamen; |
| DA | dopamine; |
| DOPAC | 3,4-Dihydroxyphenylacetic acid; |
| HIP | Hippocampus; |
| HVA | Homovanillic acid; |
| pFC | preFrontal Cortex; |
| NAc | Nucleus Accumbens; |
| NE | Norepinephrine; |
| 5-HIAA | 5-hydroxyindoleacetic acid; |
| 5-HT | Serotonin, |
| WT | Wild-type |

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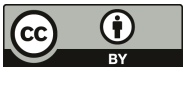

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# How Children with Autism Spectrum Disorder, Developmental Language Disorder, and Typical Language Learn to Produce Global and Local Semantic Features

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Received: 27 February 2020; Accepted: 9 April 2020; Published: 11 April 2020

**Abstract:** A local processing bias, often considered a cognitive style unique to autism spectrum disorder (ASD), may influence the types of semantic features acquired by children with ASD and could contribute to weaknesses in word learning. Children with developmental language disorder (DLD) also struggle to learn semantic aspects of words, but this cognitive style has not been ascribed to children with DLD. The purpose of this study was to explore whether global–local processing differences influence the type of semantic features children with ASD, DLD, and their neurotypical peers learn to produce when learning new words. Novel word definitions produced by 36 school-aged children (12 with ASD, 12 with DLD, and 12 with typical language) who participated in an extended word-learning paradigm were used to extract newly learned semantic features. These semantic features were then coded for global and local attributes and analyzed to detect whether there were differences between groups. Results indicated that the children with ASD and DLD produced more global, rather than local, semantic features in their definitions than the children with typical language. An over-reliance on global, rather than local, features in children with ASD and DLD may reflect deficits in depth of word knowledge.

**Keywords:** autism spectrum disorder; developmental language disorder; semantic features; word learning; central coherence

## 1. Introduction

Currently, there are ongoing conversations over whether autism spectrum disorder (ASD) and developmental language disorder (DLD) are different ends on a continuum of the same disorder [1–3]. Shared traits and similar performance on language tasks perpetuate this discussion. For instance, children with ASD perform poorly on the nonword repetition task [4], a hallmark weakness for children with DLD [5]. Although DLD is primarily characterized by deficits in morphosyntax, tense marking is also impacted in children with ASD [4,6]. Pragmatic deficits are a clinical marker for ASD, but children with DLD can display social communication weaknesses as well [7]. This overlap leads practicing clinicians to report that ASD and DLD can make for a "difficult differential diagnosis" [8]. This challenge is exacerbated when children with DLD also meet the clinical standards for a diagnosis of ASD on the social or communication domains of the Autism Diagnostic Interview, Revised (ADI-R) or the Autism Diagnostic Observation Schedule (ADOS [9]) or both [10].

Efforts to uncover distinct patterns of errors on these language tasks have made some headway in identifying key differences between ASD and DLD. For example, specific patterns of error have been found between groups of children with ASD and DLD on the nonword repetition task [11]. Even though morphosyntactic deficits have been reported in children with ASD, these errors may not

include the morphological omission errors that are characteristic of DLD [12]. In a comprehensive review by Williams, Botting, and Boucher [13], further distinctions are described in great detail, such as the widespread phonological deficits in DLD but not in ASD (however, phonological short-term memory deficits have been found in both disorders [14]). These efforts to distinguish between ASD and DLD are essential to elucidate unique language profiles that could aid in earlier and more accurate differential diagnosis.

These challenges in distinguishing between ASD and DLD persist even after the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) revisions were designed to improve accuracy of diagnoses. For a diagnosis of ASD, deficits in social communication and restricted or repetitive behaviors must be present [15]; however, neither of these deficits is necessary for a diagnosis of DLD. Furthermore, ASD must be ruled out to meet the criteria for DLD. As defined by Leonard [16], DLD s a "significant deficit in language ability" for one's chronological age not caused by hearing loss, nonverbal intelligence, or other neurological deficits (p. 3). Moreover, both groups often perform similarly on tasks outside of the language domain, such as on tasks of motor skill [17]. Because commonalities between ASD and DLD exist, clinicians are often forced to rely on areas known not to overlap, such as restricted or repetitive behaviors, to make a differential diagnosis.

With this high degree of symptom overlap, it is possible that global–local processing differences may be used to help differentiate these two disorders. Individuals with ASD are described as having a cognitive style that lends itself to local processing more than gestalt, or global processing [18–20]. This cognitive style is labeled as weak central coherence, or the reduced ability to pull information "together for higher-level meaning" [19,21,22]. This local processing bias is a tendency to focus on small details rather than larger, or more global contexts [19]. In the linguistic domain, this difficulty, i.e., "seeing the forest for the trees", impacts one's ability to engage in everyday tasks, such as following along with a story [23–25] or applying a shape cue when learning words [26–29]. Although global–local processing has been widely measured in individuals with ASD, it has been less frequently, or at least more indirectly [30–33], assessed in children with DLD. When it has been explored, children with DLD have not consistently shown a global or local preference [2,30].

Understanding how children with ASD handle global and local information during tasks of word learning is paramount to developing more effective language interventions. For example, in typical development, toddlers quickly recognize that objects with the same global shape have the same word label [34]. By 24 months of age, these children apply this global shape cue to extend word labels more readily than local cues, such as texture or color [35]. However, this facilitative "shape bias" cue based on global processing has not been found in young children with ASD [26–28] or in school-aged children with ASD who have been described as low-functioning [29], showing how the prioritization of local over global processing may contribute to the deficits in word-learning often reported in children with ASD [8,36,37]. Differences in global and local processing also may impact which relevant semantic features of words children with ASD acquire as they form abstract mental representations, or prototypes, of words in their memory. Typically developing infants utilize these abstract prototypes for early categorization [38], and these prototypes are often based on the global shape cue because shape is the most pertinent cue for early object categorization. Perhaps, then, it is unsurprising that children with ASD do not apply abstract prototypes on word categorization tasks [39] or word fluency tasks [40] if they do not attend to pertinent global semantic cues.

Although global shape cues are valuable for early word learning, acquiring the local, detail-specific semantic features of words as children build semantic representations in their mental lexicon is also a fundamental step in developing more complex aspects of language, such as recognizing the salient aspects of words, understanding multi-meaning words, forming sentences, using figurative language and humor, and producing narratives, all areas of difficulty for children with ASD [23–25,39–42]. As children establish semantic representations of words, global semantic features could reflect word referents as a whole, such as describing a cow (basic level) as an animal (superordinate level) or as a heifer (subordinate level). Local semantic features may pertain to a part or detail of the word referent,

as in describing a zebra as having stripes. Distinctive semantic features have been shown to aid successful word retrieval in typical learners [43]. However, children with ASD have been reported to acquire fewer semantic features on word-learning tasks than their typical peers [44] and, for those with concomitant syntactic deficits, show sparser word knowledge [8], which may further hinder their ability to successfully produce words. Discovering facilitative ways to teach children with ASD new words seems especially impactful for improving their quality of life, considering that nearly 20% of children with ASD produce fewer than five words on a given day [45]. For clinicians, knowing how children with ASD and DLD acquire global and local semantic features would inform how best to teach new words in intervention, which could have diffuse benefits in their overall language comprehension and use. However, to date, no study has explored how children with ASD and DLD learn to produce global and local aspects of words.

### 1.1. Global–Local Processing in ASD

Performance consistent with the weak central coherence hypothesis has been observed in individuals with ASD on verbal [18,24,25,46–48], as well as non-verbal [49–54] tasks. In fact, some have suggested that this local bias is a core component of the ASD phenotype [55,56]. Because the weak central coherence hypothesis proposes this cognitive style impacts those with ASD, regardless of age, intelligence, and language ability [19,46,57], global–local processing differences may serve as a potential way to bypass the language commonalities often observed across ASD and DLD to help successfully differentiate between these two disorders.

Local biases influence language productions in ASD. Although this global–local difference has primarily been observed at the level of processing, it is important to determine whether there is any impact on the language productions of individuals with ASD. In a study by Fitch, Fein, and Eigsti [18], adolescents with and without ASD were asked to describe oil paintings by famous artists under a cognitive load (tapping with an index finger). The group with ASD produced as many global details as the other groups; however, the adolescents with ASD still made more local observations than those with typical development, as well as adolescents who had overcome an earlier ASD diagnosis (i.e., optimal outcome; for more information on optimal outcome in children with ASD, see [58,59]). The local bias was apparent in individuals with ASD during this language production task as well.

Booth and Happé [57] utilized a sentence completion task to compare local biases in children and young adults with ASD, typical language development (TLD), and attention deficit hyperactivity disorder (ADHD). On this task, individuals were asked to finish a sentence prompt (e.g., In a cave lived a bat and...), and then their responses were coded as either showing global integration of the over-arching sentence meaning (i.e., a response such as bear or spiders) or local biases (i.e., a response such as ball). Using this language production task, the individuals with ASD were more likely to produce a response with a local bias than their age- and IQ-matched typical peers, as well as their peers with ADHD (to rule out executive function/inhibitory skills as a contributing factor to locally-biased responses). Language production tasks may be used to uncover the local processing bias proposed to reflect weak central coherence in individuals with ASD.

### 1.2. Global–Local Processing in DLD

Although global–local processing in children with ASD has been extensively studied, less is known about global–local processing in children with DLD. To determine if children with DLD have visuo-spatial processing deficits specific to local and global processing, Akshoomoff, Stiles, and Wulfeck [30] compared the performance of children with DLD and typically developing children on the Hierarchical Forms memory task and the Rey–Osterrieth Complex Figure (ROCF) task. The Hierarchical Forms task required the participants to examine visual stimuli constructed in such a way that a larger symbolic image is made up of many smaller symbols that differed from the larger symbol. On this task, the children with DLD were less accurate than the typically developing group overall, but the groups did not differ in accuracy with respect to global and local levels. The authors concluded

that the children with DLD, "may adopt simpler or more immature processing strategies . . . but global or local processing would not be selectively affected" [30].

The results for the ROCF task were similar to the Hierarchical Forms task. The ROCF task required the groups to reproduce a drawing from memory, and performance on this task is known to correlate with visuospatial processing abilities. The children in the DLD group drew fewer details, less accurate figures, and more incorrect cluster placement than the control group on the ROCF task. The authors concluded that the children with DLD relied on a less accurate, immature strategy when copying the figure. Even though these findings exemplify a different pattern of visuo–spatial processing in children with DLD, their performance did not directly reveal differences in global–local processing from their typical peers [30]. If individuals with DLD process global and local information typically (albeit more immaturely), global–local processing tasks may be a viable way to clinically differentiate between ASD and DLD.

### *1.3. Comparing Global–Local Processing in Children with ASD to those with DLD*

Global–local processing on linguistic tasks in children with ASD compared to those with DLD has led to mixed findings. In one study, Norbury [31] administered a lexical ambiguity task. In this task, words with ambiguous meanings (e.g., bank) were embedded in sentences given to children with ASD and typical language, ASD and language impairment, DLD, and TLD who had to use context clues to determine which meaning was appropriate (e.g., John stole from the bank). Participants were then shown a picture that was either congruent or incongruent with the meaning best reflected in the sentence and asked to respond "yes" or "no" if the picture matched. According to the weak central coherence hypothesis, individuals with ASD, regardless of language abilities, should show difficulty extracting meaning from broader contexts [19,46]. However, language ability, rather than autism spectrum status, was a better indicator of performance on this task. This well-designed study provides some evidence that the challenges observed in individuals with ASD often attributed to weak central coherence may be better explained by deficits in lexical and semantic knowledge [31].

More recently, Riches and colleagues [32] explored whether autism status or language ability better reflected weak central coherence using a similar forced-choice syntactic ambiguity task with adolescents with ASD and typical language, ASD and language impairment, DLD, and TLD. Unlike the Norbury [31] findings, neither autism status nor language ability led to any significant differences in performance on this linguistic processing task. However, because both studies administered a forced choice task, it is possible that the use of a more open-ended approach would have led to different outcomes.

Although not intended to be a comparison between subgroups of children with ASD with and without language impairments, the open-ended Sentence Completion Task utilized by Booth and Happé [57] included children with autism and children with Asperger syndrome based on the DSM-IV diagnostic criteria, which included a history of spoken language delay for a diagnosis of Autistic Disorder but required an absence of developmental language delay for a diagnosis of Asperger's Disorder. In this study, both groups of children showed local biases compared to their age- and IQ-matched peers, providing some evidence that autism-status, rather than language or IQ, plays a more influential role in whether or not a child will demonstrate a local-bias on an open-ended, linguistic production task.

In summary, weak central coherence might be a differentiating characteristic between children with ASD and those with DLD. To capture these global–local processing differences, previous studies have primarily employed standardized assessments [30,49,53], magnetic resonance imaging [51,60], switching tasks [54], and scripted sentences or stories followed by a forced choice set of answers [31,32], none of which use the open-ended approach recommended by Happé [22] to best evoke differences in global–local processing. Unlike a labeling, forced choice, or recognition task, open-ended production tasks require the participant to formulate his or her own answers. If global–local processing differences

exist between children with ASD and those with DLD, an open-ended task would likely best elicit these differences.

### *1.4. Research Question*

In the current study, we embarked on a more open-ended approach. This investigation aimed to explore whether differences in the production of global and local semantic features in a definition task of newly learned, novel words could be used to differentiate children with ASD from those with DLD and TLD. Additionally, knowing how these intrinsic-to-the-learner processing differences impact how children acquire new words is a vital component in better facilitating language learning in these populations. Because children with ASD show a bias toward local details when processing new information, we predicted that they would produce more local semantic features than their peers with DLD and TLD during a novel word definition task; the children with DLD and TLD were expected to produce similar amounts of local and global semantic features.

## 2. Materials and Methods

To explore how children produce global and local semantic features of newly learned words, data collected during previously conducted novel word-learning studies in children with DLD and TLD [61] and with ASD [62] were used for the current study. These original word-learning studies investigated the influence of enriched semantic input on the ability of children with ASD, DLD, and TLD to learn novel words over time. This same data set has also been used to compare how children with ASD, DLD, and TLD acquire visually and verbally presented semantic features during tasks of novel word-learning [63]. In the current study, these novel word definitions were used to determine if the production of global and local features differed by group, potentially shedding light on how local-processing biases influence word-learning in ASD. All of the original recruitment and experimental procedures implemented in the novel word-learning investigations, as well as the analytic procedures and data management for the current study, adhered to the ethical standards approved by each university's ethical review committee.

### *2.1. Participants*

To determine the appropriate sample size for the current study, G*Power statistical software [64,65] was used to conduct a power analysis. For this power analysis, an alpha level of 0.05, power of 0.80, and a moderate effect size of 0.25 were entered as the set parameters for a repeated measures ANOVA with the within (three processing levels) by between (three groups) interaction designated as the planned statistical test. This analysis indicated that a minimum total sample size of 36 would be sufficient. Thus, data from 36 children, 12 children with ASD, 12 children with DLD, and 12 children with TLD, from the original word-learning studies were used for this follow-up study exploring global–local feature productions. All children were recruited from Tippecanoe County, Indiana, USA, and its surrounding counties. For inclusion in the original studies, all participants must have passed an oral-mechanism examination, showed hearing within normal limits on a bilateral pure tone hearing screening, achieved a standard score of 85 or higher on a nonverbal IQ test, and were monolingual English speakers.

Because the previous and current investigators were primarily interested in the production, rather than the comprehension, of newly learned semantic features, and because expressive vocabulary is more reliably measured than receptive vocabulary in children with ASD [66], the expressive vocabulary of each group was compared using raw scores from the Expressive Vocabulary Test-II [67] to ensure the groups did not significantly differ on this key measure (see Table 1). Consistent with previous work indicating that expressive vocabulary is an area of weakness in children with ASD [37] and DLD [68], this matching procedure led to a group with TLD who was significantly younger than the groups with ASD ($p < 0.01$) and DLD ($p = 0.04$). Because the number of locally-biased responses on open-ended production tasks of central coherence has not been shown to differ based on age [57], the data from this

original TLD group were still included for comparison. The two clinical groups (ASD and DLD) did not significantly differ in age from each other ($p = 0.17$). Also, because children with ASD show relatively greater impairment in comprehension than production [69], a paired samples *t*-test was conducted to check for differences between expressive and receptive vocabulary in these children. A paired samples *t*-test comparing standardized scores on the Expressive Vocabulary Test—2nd Edition (EVT-2) and the Peabody Picture Vocabulary Test-4 [70] did not reveal any significant differences between receptive (M = 98.42, SD = 18.96) and expressive vocabulary (M = 95.75, SD = 7.57) in the children with ASD, $t(11) = -0.56$, $p = 0.59$. Table 1 depicts a summary of the participant characteristics in all three groups.

**Table 1.** Summary of the participant characteristics.

| | ASD ($n$ = 12) *M* (Range) | DLD ($n$ = 12) *M* (Range) | TLD ($n$ = 12) *M* (Range) | *F* Value | *p* Value |
|---|---|---|---|---|---|
| Age (years; months) | 7; 9 (4; 6–11; 3) | 7; 1 (5; 9–8; 4) | 5; 10 (4; 3–7; 3) | 6.39 | 0.01 |
| Sex | 3 F, 9 M | 3 F, 9 M | 6 F, 6 M | 1.10 | 0.34 |
| EVT-2 Raw Score | 88.67 (53–120) | 82.00 (67–97) | 94.5 (68–128) | 1.41 | 0.26 |
| EVT-2 Standard Score | 95.75 (79–112) | 94.17 (78–106) | 114.83 (91–135) | 15.66 | <0.01 |
| Nonverbal IQ Standard Score | 96.6 (85–106) * | 104.08 (91–125) | 121.50 (96–149) | 12.88 | <0.01 |
| Language Standard Score | 86.18 (58–111) * | 73.67 (42–87) | 112.09 (90–125) | 21.63 | <0.01 |

EVT-2 = Expressive Vocabulary Test—2nd Edition; F = female, M = male; ASD = autism spectrum disorder; DLD = developmental language disorder; TLD = typical language development; Nonverbal IQ Standard Scores were from the Primary Test of Nonverbal Intelligence, the Columbia Mental Maturity Scale, or the Test of Nonverbal Intelligence; Language Standard Scores were from the Structured Photographic Expressive Language Test- Preschool–2nd edition, Structured Photographic Expressive Language Test–3rd edition, or the Clinical Evaluation of Language Fundamentals–4th edition; *, only includes scores from 11 participants with ASD. One-way ANOVA with equal variance assumed for statistical comparisons.

The children with ASD were initially recruited for a study exploring the role of semantic richness in word-learning in these children [62]. For inclusion in this original study, the participants with ASD must have a reported independent medical diagnosis of ASD. Then, as part of the inclusionary testing, a trained clinician administered the Autism Diagnostic Observation Schedule—2nd edition [71] to each participant with ASD to confirm that they met the cut off scores for either autism or the autism spectrum. All of the children with ASD included in the original studies were verbal communicators who did not use any form of augmentative or alternative communication as a primary means of communication. Following these inclusionary testing procedures, 12 children (three females) with a mean age of 7; 9 (years; months, range 4; 6–11; 3) were included with ASD. One participant (ASD1) was unable to complete the nonverbal IQ test due to a behavioral rigidity that led to the consistent selection of items in the same location from the array of choices. Because ASD1 was able to successfully participate in the experimental word-learning tasks, her expressive vocabulary score was similar to participants with DLD and TLD, weak central coherence is not hypothesized to depend on intelligence [19], and intelligence has not been shown to be a significant factor on open-ended tasks exploring central coherence [57], her data were still included in the current study. After meeting all inclusionary criteria, the Structured Photographic Expressive Language Test—Third Edition [72] or the core battery of the Clinical Evaluation of Language Fundamentals—4th Edition [73], whichever was age appropriate, was administered to eleven of the children with ASD to capture their broader expressive language skills. Due to time constraints, one participant with ASD was not given either expressive language test.

The children with DLD and TLD were originally recruited to participate in a multi-year, longitudinal study exploring the relationships between motor and language skills [61,74–77]. As such, the inclusionary testing procedures for these children were implemented one or two years before the collection of their novel word definitions that were used for comparison in the current study. Inclusion criteria outlined by Leonard [16] were used when qualifying participants for the group with DLD. Specifically, these participants obtained scores at or above 85 on a standardized nonverbal IQ test, demonstrated hearing and oral-mechanism functioning within normal limits, and had no

history of a neurological disorder. Additionally, during their initial year in the longitudinal motor and language investigation, each participant achieved a standard score at or below 87 on the Structured Photographic Expressive Language Test–Preschool—2nd edition [78], which has good sensitivity and specificity when diagnosing DLD [79] using the criteria outlined by Greenslade, Plante, and Vance [80]. Finally, to rule out ASD, all children with DLD were assessed with the Childhood Autism Rating Scale—2nd Edition [81] and secured scores within the "Minimal-to-No symptoms" range. Based on these inclusionary criteria, 12 children (three females) with a mean age of 7; 1 (range 5; 9–8; 4) were included with DLD in the current study.

To be included in the group with TLD in the original longitudinal study, parental reporting was used to confirm that the children had no history of language delays. Also, the children had to have achieved a standard score of 85 or higher on either the Structured Photographic Expressive Language Test–3rd edition [72] or the core battery of the Clinical Evaluation of Language Fundamentals–4th edition [73], depending on which was age appropriate at the time of their initial inclusion in the longitudinal study. Finally, all children with TLD received scores within the "Minimal-to-No symptoms" range on the Childhood Autism Rating Scale—2nd edition [81]. Based on these inclusionary procedures, 12 children (six females) with a mean age of 5; 10 (range 4; 3–7; 3) with TLD were included in the current study.

### *2.2. Auditory Stimuli*

Six novel words (/fʌʃpəm/, /pʌvgəb/, /bʌpkəv/, /mʌfpəm/, /fʌspəb/, and /pʌbtəm/) were presented auditorily to the children in the original word-learning studies [61,62]. These two-syllable phonetic strings were controlled for phonotactic probability and neighborhood density, factors known to affect a word's learnability [82,83]. All words were recorded by a female native-English speaker and loaded into Praat [84] to equate for intensity at 70 dB Hearing Level. The novel words were presented through a set of external speakers located in front of the participants. Depending on the original semantic cue condition (no semantic cues, sparse semantic cues, or rich semantic cues [62]), recordings of four of these novel words were presented in synchrony with a matched visual referent (i.e., paired word form with meaning) either in isolation (sparse semantic cues condition) or embedded in a children's story (rich semantic cues condition). Two novel words were never paired with visual-referents (no semantic cues condition) to compare how children produce words given semantic cues to those taught without any semantic information. Only the novel words taught with visual referents (i.e., sparse and rich semantic cues conditions) were included in the current study. All three pairs of novel words were randomized and counterbalanced across participants and groups.

### *2.3. Visual Stimuli*

In the original word-learning studies, four child-friendly drawings by a professional illustrator (Figure 1) were used as the visual referents for the novel words [61,62]. Each visual referent came from a distinct superordinate semantic category; a tool, an instrument, an animal, and a vehicle. In the original studies, the tool and instrument referents were taught in the sparse semantic cue condition. In this sparse semantic cue condition, the children were auditorily presented a novel word in synchrony with the visual referent. For the animal and vehicle referents, the novel words were embedded in a children's story in the rich semantic cue condition. Prior to teaching these visual referents in the semantically enriched condition in the original word-learning studies [61,62], all four visual stimuli were tested in the semantically sparse condition to assess whether any image was inherently more learnable. Based on this testing, no referent was significantly more learnable in any of the original word-learning measures (e.g., referent identification, confrontation naming, phonetic accuracy, or kinematic stability). All visual images were displayed on a 76.2 cm Dell monitor screen placed in front of the children that was connected to a laptop with Microsoft PowerPoint. The children's story script with all of the corresponding visual images is available in Gladfelter and Goffman [62] and is provided in the Supplementary Materials for this article.

**Figure 1.** Visual referents used in the original word-learning paradigm [62].

### *2.4. Collection of Word Definitions*

The definitions used to extract local and global semantic features were collected following their presentation in either the sparse semantic cues condition (i.e., picture–word pair in isolation) or the rich semantic cues condition (i.e., embedded in a children's story) in the original word learning studies [61,62]. To control for any primacy or recency effects, the presentation order for the three semantic learning conditions (no semantic cues, sparse semantic cues, rich semantic cues) was counterbalanced across children. These original studies focused on whether the semantic richness of the learning context influenced a child's ability to acquire new words, whereas the current study expands upon this earlier work by exploring the differences in the types of semantic features the children produced, specifically at the global or local processing level.

In these prior studies, participants were presented novel words seven times on three separate days approximately one week apart (or 21 total exposures per novel word across all sessions). After being presented with the meanings of the novel words in each semantic cue condition, participants were asked to define the novel words using the open-ended examiner prompt, "What does ____ mean?". After their initial response, all participants received one follow-up prompt, "What else can you tell me about _____?" [85]. These open-ended prompts are unlike some past studies targeting global–local processing (e.g., [31]), which limited their participants to two choices (e.g., "yes" or "no"). Although the original word learning studies were not explicitly designed to target central coherence, open-ended tasks are recommended for assessing the impact of global–local processing in children with ASD [22], making the use of these novel word definitions an ideal method for comparing global and local productions in children with ASD, DLD, and TLD. All definitions were recorded and transcribed for later coding. A total of 432 definitions (36 participants × 4 definitions × 3 sessions) from these word-learning studies provided the data for the current study.

### *2.5. Extraction of the Semantic Features from the Definitions*

In the original word-learning studies, the semantic features were extracted from the definitions to score the number of accurate units of information (i.e., the number of semantic features) drawing from the method described in McGregor, Sheng, and Ball [85]. As an example, one child defined the vehicle as follows: "In the story, Big Brother said his /pʌbtəm/ makes donuts [1]. He said it's shiny [2], and it looks like a motorcycle [3] and it goes faster [4] and faster!". This definition contained four accurate units of information about the meaning of the target word. In the original investigation, a second coder was trained to calculate the reliability of the number of accurate units of information produced. For reliability training, the definitions from three randomly selected participants (one from each diagnostic group) were scored separately by both coders for the number of accurate semantic

features. Then, within the context of training, disagreements were thoroughly discussed, and consensus building took place. For the reliability scoring, a new set of definitions distributed equally across groups from 25% of all sessions was selected using the same random number generator (random.org) to select the participant numbers. The total number of semantic features identified by the original primary author (Gladfelter) was 270 and by the second coder was 284, with an overlap of 269 semantic features. Reliability was then judged to be between 94.7% (269/284) and 99.6% (269/270). For the current study, the semantic features from all 432 definitions were analyzed based on whether the semantic information was a global or local attribute.

### *2.6. Global and Local Coding of Semantic Features in the Current Study*

The semantic features extracted in the original word-learning studies were used in the current study. To prevent bias during coding, the second author was blinded to the diagnostic category of each participant using a de-identifying alphanumeric coding system devised by the first author. A coding manual was designed to promote consistency across coders and to explain the coding process to an undergraduate research assistant for later reliability coding. The second author used a Microsoft Excel worksheet to code the participants' definitions following manualized rules developed by the authors.

The semantic features were analyzed to see if they reflected a local detail or the global object. Although previous word-learning and categorization studies have used the global shape cue to explore how children apply this category relevant cue to category irrelevant cues (e.g., size, color, or texture) when learning new words, the purpose of the current study was to focus on which semantic features produced by children required processing of the novel referent as a whole or only required the processing of local details, or smaller parts, of the novel referent as they formed semantic representations of the newly-learned words. This use of semantic features produced during a novel word-learning definition task is a new approach to investigating global–local processing. The weak central coherence hypothesis [19,21] proposes that children with ASD show a processing bias for local details at the expense of holistic meaning. This hypothesis has classically been assessed using the Navon Hierarchical Figures Task [86], which presents alphabetic letters composed of smaller alphabetic letters and then determines whether the individual preferentially processes the local parts (smaller letters) or the global whole (bigger letters) of a visual image. Using hierarchical figure tasks, individuals with ASD have been shown to demonstrate a preference for local, rather than global processing, the opposite pattern of those with more typical development [87,88]. To more closely align with this classic global–local task, rather than a word categorization task, we chose to code semantic features that either captured the novel word-referent as a global whole object or as a local part.

To analyze the processing level, the coders determined if each semantic feature was (1) Global (whole object), (2) Local (details or parts), or (3) N/A, indicating coding was not applicable at the global or local level. If the participant provided a semantic feature that described the target referent as a whole, the coders scored it as Global (whole object), or, if the participant produced a semantic feature that described a part or detail of the target referent, the coders scored it under the Local (details or parts) category. For example, if the child said "antennas" for the animal target referent, it was coded under Local because this pertained to a specific attribute of the animal and not the whole. If the child produced a semantic feature such as "pet," it was marked as Global because it referred to the whole referent. It is worth noting that the global–local coding implemented in the current study was conducted on each of the originally extracted semantic features individually and not on all features provided within a definition collectively. In other words, if a child's definition provided several detail-specific features that, together, would provide a more holistic description of the referent, these individual features were still coded as Local.

Not every semantic feature was marked for local or global processing because not all semantic features were able to be coded as a global or local attribute (e.g., the semantic feature was an action, emotion, or descriptive word). In this case, the coder scored the semantic feature as N/A for not

applicable. For example, the coder marked "N/A" if the child said "gives kisses" to define the animal referent because it could not be separated into global or local parts.

### 2.7. Reliability and Training

To assess the inter-rater reliability of the global/local semantic feature coding, one undergraduate research assistant majoring in Communicative Disorders coded 25% of the definitions (i.e., data from nine participants). These were chosen using a random number generator (www.random.org) to select the participant numbers, with an equal distribution across the three diagnostic groups. The selection of 25% of the total data collected fits within the criteria outlined by Schlosser [89], which recommends inter-rater reliability be conducted between 20%–30% of the total data. The randomly selected set of participants used for the final reliability coding did not include any data used during reliability training and was also de-identified using the same alphanumeric system to blind the undergraduate coder and the second author of each participants' diagnostic category. To determine inter-rater reliability, Cohen's kappa was derived before consensus building occurred. Following the ratings described by Hallgren [90], the kappa statistic for the processing-level coding was almost perfect agreement (k = 0.932 with a 95% confidence interval of 0.881–0.983). Disagreements were discussed, and then consensus building took place.

### 2.8. Statistical Analyses

A mixed-model ANOVA was conducted with diagnostic group (ASD, DLD, and TLD) as the between-subjects variable, and processing level (global vs. local vs. not applicable) served as the within-subjects variables. From the original 432 definitions, a total of 817 semantic features, with 257 from the children with ASD, 335 from the children with DLD, and 225 from the children with TLD, were coded. The sum of semantic features within each global, local or N/A coding category was calculated individually for each participant and collapsed across sessions. For the mixed-model ANOVA, these summed totals of responses served as the within-subjects data. An alpha level of less than 0.05 was considered significant.

## 3. Results

This study aimed to determine whether the global or local semantic features produced during a definition task could be used to differentiate children with ASD from those with DLD and TLD. A summary of the results for diagnostic group and processing level effects is presented in Table 2.

**Table 2.** Summary of ANOVA for Group, Processing Level, and Session Effects.

| Effect | *F*-Value | *df* | *p*-Value | Partial Eta Squared |
|---|---|---|---|---|
| Group | 1.27 | 2, 33 | 0.295 | 0.07 |
| Processing Level | 26.21 | 2, 32 | <0.001 * | 0.62 |
| Processing Level by Group | 2.86 | 4, 66 | 0.030 * | 0.15 |

*df* = degrees of freedom. * indicates significance at the 0.05 alpha level.

### 3.1. Global–Local Processing Level Effects

The mixed-model ANOVA revealed a significant effect based on the global–local processing level ($p < 0.001$). Follow-up least significant difference (LSD) pairwise comparisons indicated that more global than local ($p < 0.001$) semantic features were produced during the novel word definitions. Also, more features were categorized as N/A than as global ($p < 0.001$) or local ($p < 0.001$). Because the primary goal of this study was to assess the influence of global and local processing on the production of semantic features, this significant finding is not further discussed here.

### *3.2. Group and Global–Local Processing Interaction Effects*

Although the mixed-model ANOVA did not reveal a significant group effect ($p = 0.295$), it did reveal a significant interaction between diagnostic group and processing level ($p = 0.030$). Follow-up pairwise comparisons (LSD) indicated that the children with DLD produced significantly more global semantic features than their peers with TLD ($p = 0.012$), and the children with ASD approached significance ($p = 0.054$) towards producing more global semantic features than their peers with TLD. The groups with DLD and ASD did not differ from each other ($p = 0.522$) in their production of global semantic features. There were no other significant interactions between groups and local semantic features or features coded as N/A (all $p$ values >0.05).

Within each group, the children with ASD ($p = 0.008$) and DLD ($p < 0.001$) produced significantly more global features than local features within their novel word definitions. The children with TLD did not differ in their production of global and local semantic features ($p = 0.877$). All groups of children produced more N/A features than global and local semantic features (all $p$ values < 0.05), except for the children with ASD who did not differ in their production of global and N/A features ($p = 0.224$). Because the study aimed to focus on global and local semantic features, these significant N/A findings are not further discussed here. All group means and standard deviations for each processing level are summarized in Table 3, and each participant's mean number of features is presented in Table 4.

**Table 3.** Group semantic feature descriptive statistics by processing level.

| Processing Level | Group | Mean | SD | Min | Max |
|---|---|---|---|---|---|
| Global | ASD | 7.50 | 4.78 | 0 | 15 |
| | DLD | 8.50 | 3.94 | 1 | 17 |
| | TLD | 4.42 | 2.15 | 0 | 8 |
| Local | ASD | 3.00 | 3.25 | 0 | 9 |
| | DLD | 2.33 | 3.92 | 0 | 14 |
| | TLD | 4.17 | 5.44 | 0 | 17 |
| NA | ASD | 10.92 | 8.77 | 0 | 28 |
| | DLD | 17.08 | 12.80 | 0 | 40 |
| | TLD | 10.17 | 8.16 | 0 | 24 |

SD = standard deviation.

**Table 4.** Mean number of semantic features for each participant for each processing level.

| Participant | Global | Local | NA | Participant | Global | Local | NA | Participant | Global | Local | NA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **ASD01** | 4.00 | 0.00 | 0.00 | **DLD01** | 4.00 | 0.00 | 12.67 | **TLD02** | 1.67 | 4.00 | 7.67 |
| **ASD02** | 3.33 | 1.00 | 3.67 | **DLD04** | 1.33 | 0.00 | 5.67 | **TLD03** | 1.33 | 2.33 | 4.67 |
| **ASD03** | 1.00 | 3.00 | 2.67 | **DLD05** | 2.33 | 0.00 | 4.33 | **TLD04** | 2.67 | 0.33 | 0.33 |
| **ASD04** | 3.33 | 2.67 | 9.33 | **DLD06** | 2.67 | 4.67 | 13.33 | **TLD06** | 2.00 | 1.00 | 2.33 |
| **ASD05** | 0.00 | 0.00 | 0.00 | **DLD07** | 2.67 | 1.00 | 6.67 | **TLD07** | 0.00 | 0.00 | 0.00 |
| **ASD06** | 5.00 | 1.67 | 7.33 | **DLD09** | 11.00 | 1.00 | 4.00 | **TLD08** | 1.67 | 1.33 | 5.33 |
| **ASD07** | 2.67 | 1.67 | 7.00 | **DLD14** | 3.00 | 0.67 | 9.00 | **TLD09** | 1.33 | 5.67 | 4.00 |
| **ASD09** | 4.00 | 1.33 | 4.33 | **DLD17** | 5.67 | 0.67 | 6.00 | **TLD11** | 1.33 | 1.67 | 8.00 |
| **ASD10** | 1.00 | 0.00 | 1.33 | **DLD18** | 3.00 | 0.67 | 5.00 | **TLD12** | 1.00 | 0.33 | 2.67 |
| **ASD11** | 0.33 | 0.00 | 3.00 | **DLD19** | 2.67 | 0.00 | 3.33 | **TLD13** | 2.33 | 0.00 | 2.00 |
| **ASD12** | 3.00 | 0.67 | 3.00 | **DLD20** | 2.67 | 0.00 | 0.00 | **TLD14** | 1.67 | 0.00 | 3.67 |
| **ASD16** | 2.33 | 0.00 | 2.00 | **DLD21** | 0.33 | 1.33 | 1.00 | **TLD99** | 0.67 | 0.00 | 0.00 |

### *3.3. Post-hoc Results Based on Age and Expressive Vocabulary*

Because the use of global, over local, descriptive terms during definition tasks has been shown to increase developmentally [91], and the ASD and DLD groups were significantly older than the group with TLD, a follow-up ANCOVA was conducted with age as a covariate. In this post-hoc analysis, there was no significant interaction between level of processing and age, $F(2, 31) = 1.08$, $p = 0.352$.

Furthermore, because some previous studies have reported that language, rather than autism status, is a better predictor of performance on tasks assessing weak central coherence [31], an additional follow-up ANCOVA was conducted with EVT-2 standard scores as a covariate. As with the age results, this post-hoc analysis revealed no significant interaction between level of processing and language performance on an expressive vocabulary test, $F(2, 31) = 0.60$, $p = 0.553$.

## 4. Discussion

Global–local processing differences influenced the type of semantic features produced by children with ASD and with DLD compared to their typical peers on a word learning task, but not in the ways expected. It was predicted that the group with ASD would provide more local features than the group with TLD, and the group with DLD would be similar to the group with TLD in its use of global and local features. However, the groups with DLD and ASD (albeit only approaching significance) both produced more global features than the TLD group. Although these findings were unexpected within the framework of the weak central coherence hypothesis, these outcomes are consistent with a growing body of semantic learning literature [8,44,68,92–96] in children with ASD and DLD, indicating that these children show difficulty acquiring more detail-specific information. These results also align with robust literature on the whole object assumption in early word-learning [97–99] in which children assume that object labels refer to an object as a whole rather than individual parts. Furthermore, the results are consistent with some [32], but not all [31], previous work focusing exclusively on weak central coherence in the linguistic domain.

Before interpreting these results more fully, four methodological limitations must be considered. First, because the data were extracted from already completed novel word-learning studies, and because the initial power analysis indicated that the sample size was sufficient, additional participants were not recruited for this study. Although the sample size was large enough to reject the null hypothesis, additional studies beyond this initial exploratory study are needed to replicate and more thoroughly investigate global and local processing's influence on language production tasks in children with ASD and DLD. Second, because of the original decision to match groups on expressive vocabulary, the groups with ASD and DLD were significantly older than the group with TLD. Although previous work investigating central coherence in individuals with ASD did not find any effects based on age [57] and our post-hoc analysis did not uncover any age-related effects, future research should include a chronological age-matched group with typical language to more directly determine whether developmental maturity is a contributing factor. Third, nearly half of the children with ASD also showed signs of a concomitant language disorder based on standardized language assessments. Perhaps, then, it is unsurprising that no differences were found between the children with ASD and DLD on this language production task. In one previous study, language ability, rather than autism status, was found to impact performance in comprehension tasks comparing global–local processing in children with ASD and DLD [31], suggesting that this may be a contributing factor in this production task as well. However, this finding has not been consistently replicated in later studies employing similar language comprehension tasks of global–local processing [32]. In the current exploratory study, a post-hoc analysis did not reveal any language-related effects based on expressive vocabulary scores, but clearly additional research is needed to fully assess the relationship between receptive and expressive language abilities and global–local processing in children with ASD and DLD beyond this study. Finally, because the current study analyzed already collected data, no measures of non-verbal global–local processing were implemented during the original word-learning studies for comparison to the verbal measures explored in this study. Future research that directly assesses both verbal and nonverbal global–local processing in children with DLD and ASD is necessary to fully determine the influence of verbal semantic weaknesses on tasks of weak central coherence.

### *4.1. The Local Biases in ASD Revisited*

We anticipated that the children with ASD would produce an over-abundance of local descriptor words because of their local perceptual biases; however, they unexpectedly produced a similar amount of local semantic features and a trend toward more global features than their typical peers. In hindsight, this should not have been surprising. Traditionally, evidence in support of the weak central coherence hypothesis has focused on visuo-spatial tasks [49–51,54], whereas evidence in the linguistic domain has been varied [31,32]. Previous researchers have shown that verbal children with ASD can establish semantic categories for words at the basic and superordinate levels as well as their typical peers [96], recognize typical members of familiar word–object categories [92], and can extend word label categories broadly [95], all tasks that would require them to process word referents at the global level. It is worth noting that, although children with ASD can overcome local biases to acquire globally descriptive terms when learning new words, not all children with ASD do [26–29].

One reason for this discrepancy in findings could be due to the conceptualization of central coherence. As discussed by Riches and colleagues [32], there are two differing emphases within this hypothesis; either a reduced ability to integrate information or an enhanced ability to focus on local information (p. 156). Linguistic studies more often focus on the integration side of this hypothesis, such as employing tasks that, at the local level, may be ambiguous, but when the information is integrated across the global and local levels, there is a correct interpretation and response. For example, previous work used homographs [46,47], multi-meaning words [31], or sentence fragments [57] that required the listener to pull together contextual information to select the more appropriate pronunciation, word meaning, or phrase. In contrast, studies outside of the linguistic domain focused more heavily on the enhanced processing of local details, such as through the use of the embedded figures task [49] or motion perception tasks [51]. In the current study, the children's story provided both verbal (linguistic) and visual information, allowing the children to freely rely on whichever learning strategy they naturally would to acquire the semantic features of new words. Interestingly, in a previous study using this same data set [63], these same children with ASD and DLD produced more semantic features that were originally taught in the visual images rather than through the verbal modality alone or in the visual and verbal modalities in combination. Even though both clinical groups of children relied heavily on the visual modality, which would align more closely with the enhanced local processing observed on visual-perception tasks in children with ASD, these same children instead produced more global than local semantic features, which does not provide support for the weak central coherence hypothesis.

Additional methodological differences between the current study and previous weak central coherence investigations may further explain the difference in outcomes. First, the use of child-friendly cartoons, rather than the more visually complex oil paintings used by Fitch and colleagues [18], may have facilitated global–local processing in the children with ASD. Also, the painting descriptions were collected under an increased cognitive load (finger tapping). These differences could explain how the children with ASD in the current study were able to describe the novel words in terms that demonstrated an ability to integrate the local details of the target referent into a whole.

Another key difference could be within the degree of autism symptom severity. Fitch and colleagues [18] found that the current symptoms of their participants with ASD did not relate to global and local focus, but the relative severity of autism symptoms over the lifespan did. Others have found similar symptom severity associations with weaker central coherence on non-linguistic tasks in individuals with ASD [50]. Also, in minimally verbal children with ASD, a lack of a shape bias could also reflect support for weaker central coherence in children with more severe autism-related symptoms. Perhaps the children in the current study, who were all verbal and had nonverbal IQs in the typical range, did not display as severe symptoms and therefore did not present a local bias.

Also, exposure time is a likely factor. Others have posited that individuals with ASD show global perceptual deficits due to differences in visual processing speed and require longer amounts of time to recognize objects as a whole. With additional time to analyze images, individuals with ASD accurately

integrate local signals into a global whole [50,51]. In the current study, the word-referent pairs were presented 21 times over the course of three different days roughly a week apart—possibly providing ample time for the children with ASD to process the referents at the global level.

However, an ability to overcome local biases fails to capture why the children with ASD produced more global than local features. Previously, McGregor and Bean [95] sought to determine whether local perceptual biases would lead children with ASD to extend object labels too narrowly during word extension tasks. Instead, the children with ASD who also had concomitant semantic and syntactic language difficulties had established broader word categories when a narrower, more specific category boundary would have been more appropriate. Because nearly half of the children with ASD in the current study showed signs of language weaknesses, perhaps they too acquired more broad labels for the novel words. As Norbury posited in 2005, language ability, rather than autism status, may be a better indicator of one's ability to synthesize semantically relevant, higher order information.

### *4.2. An Abundance of Global Features in Children with DLD Likely Reflects Semantic Deficits*

Surprisingly, the children with DLD produced significantly more global semantic features than the group with TLD in their novel word definitions. These global features only captured the novel objects at the most basic level of detail. As an example, one participant with DLD provided the following definition for the "tool" referent (with coded features in italics): "Bucket [1] (global). Blue [2] (not applicable), shiny [3] (not applicable). Blue. It's a tool [4] (global)". In comparison, a participant with TLD responded: "Pubtum means like it looks like a bucket [1] (global) and it has gears [2] (local) in it, and like all these wires [3] (local) and it had a spinny thing in the middle [4] (local)." Both participants provided four semantic features, but the participant with TLD provided features with a more specific level of detail, giving the semantic representation more depth, whereas the participant with DLD only gave semantic features that described the referent at a more global level.

This reliance on global terms (indicative of knowledge of breadth) over local details (indicative of knowledge of depth) in children with DLD may be that they are compensating for their sparse, less in-depth, semantic representations [68]. This interpretation was illustrated in McGregor and Appel's [94] study, in which a child with DLD produced fewer detailed, local features and instead substituted for a semantically related word at the same, whole-object hierarchical level (e.g., describing a helmet as a "hat"). Even when defining commonly used nouns, children with DLD define these concepts without much depth [93]. McGregor and her colleagues proposed that these shallower semantic representations in children with language impairments could be because they possess fewer words in their vocabularies compared to their typical peers [68,95]. With fewer words in their mental lexicons, the number of mappings between newly acquired words and words already established would be limited. If children with DLD possess fewer local, detail-level terms within their lexicon, they will continue to be limited in their ability to acquire and integrate the local features of newly learned words. These weaker, less robust semantic representations may also explain why children with DLD show difficulties extracting relevant information from broader linguistic contexts [31].

It is possible that children with DLD do not acquire these more detailed, in-depth semantic representations because of a global, rather than local, processing bias. However, children with DLD have not been shown to prioritize processing global over local information in levels of processing tasks previously [30]. Furthermore, children with DLD, much like those with ASD, show a weaker shape bias during novel object naming tasks than their typical peers [33], making this explanation of a preference for global, over local, processing unlikely.

### *4.3. The Use of Global Features during Word Definition Tasks Changes Developmentally*

Alternatively, the children with ASD and DLD, due to their older ages, may be providing a more developmentally advanced definitional form than their younger peers with TLD. The use of global terms demonstrates an ability to consolidate multiple semantic features representing the target referent and therefore is arguably a more mature form to use during a definition task. In contrast, using

multiple local details to describe one referent is more immature developmentally [91]. Skwarchuk and Anglin [91] state that superordinates indicate a mature definitional form that improves as children grow older. Because of the methodological decision for matching based on expressive vocabulary, the children with ASD and DLD in the current study were significantly older than the group with TLD, which may be why they included more global descriptor terms; it was developmentally more appropriate. Furthermore, Skwarchuk and Anglin [91] found that nouns elicited more superordinate terms in the children's definitions than verbs or adjectives. The target referents in the current study were all nouns, which also supports the use of superordinate terms. Rather than reflect a linguistic weakness, the use of global features to describe a noun on a definition task may have been more developmentally appropriate for the older children with ASD and DLD. However, the follow-up ANCOVA exploring a potential interaction between age and processing level of the coded semantic features in the current study was not found to be significant, which makes this developmental explanation for the over-use of global terms in the children with ASD and DLD less likely. However, to more directly address this possibility, future research should include a chronological-age matched sample of participants with typical language.

### *4.4. Clinical Implications*

This study contributes to a growing body of literature exploring the qualitative differences in the vocabulary knowledge of children with language impairments. Consistent with the findings of a massive undertaking by McGregor, Oleson, Bahnsen, and Duff [68] analyzing 25,681 definitions produced by school-aged children, the current results found that the children in both of our clinical groups (ASD and DLD) showed signs of limited depth of vocabulary knowledge, as shown by an overuse of global, rather than detailed terms, when defining new words. Further, based on the findings of the study by McGregor and colleagues [68], the older ages of the participants in our study, and work including young adults with specific learning disabilities [100], these semantic deficits persist over time. Even though clinicians often focus on pragmatic language skills in children with ASD and morphosyntactic skills in children with DLD, semantic deficits must also be addressed.

## 5. Conclusions

This study explored whether local processing biases in a word definition task in children with ASD could differentiate them from children with DLD and TLD. When acquiring local and global information, the children with ASD and DLD produced more global semantic features in their definitions compared to children with TLD. This finding does not support the idea that a local processing bias prevents children with ASD from successfully acquiring global semantic information as they learn new words. Because the children with DLD were not expected to show differences from their typical peers in global–local processing, it is unclear whether these global semantic feature production differences are due to global–local processing challenges or simply reflect weaker semantic (depth of word knowledge) skills. Future work is needed to investigate the relative contributions of global–local processing and semantic language skills in the formation of semantic representations during tasks of word-learning in children with ASD and DLD.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-3425/10/4/231/s1, Children's story script with all of the corresponding visual images.

**Author Contributions:** Conceptualization, A.G. and K.L.B.; methodology, A.G. and K.L.B.; formal analysis, A.G.; investigation, K.L.B.; resources, A.G.; data curation, A.G. and K.L.B.; writing—original draft preparation, A.G. and K.L.B.; writing—review and editing, A.G and K.L.B.; visualization, A.G.; supervision, A.G.; project administration, A.G.; funding acquisition, A.G and K.L.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** Initial data collection was supported by the National Institute on Deafness and other Communication Disorders (NIDCD) grants R01DC004826 (PI: Lisa Goffman) and 2T32DC000030 (PI: Laurence Leonard) at Purdue University. The current research analyses were supported by the Center for the Interdisciplinary Study of Language

and Literacy (CISLL) at Northern Illinois University. Funding to cover publishing costs was provided by the Northern Illinois University Libraries.

**Acknowledgments:** We would like to thank Patricia Tattersall, Janet Olson, and Lisa Goffman for their feedback throughout this project. We would also like to extend our gratitude to Alycia Tyler, Jessica Knapp, and Alyssa Bavaro. Finally, we would like to thank the participants and their families for making our research possible.

**Conflicts of Interest:** Allison Gladfelter is a salaried employee at Northern Illinois University. The authors have no other financial or non-financial conflicts of interest to declare.

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# Movidea: A Software Package for Automatic Video Analysis of Movements in Infants at Risk for Neurodevelopmental Disorders

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Received: 11 February 2020; Accepted: 30 March 2020; Published: 31 March 2020

**Abstract:** Early detecting the presence of neurodevelopmental disorders plays an important role in the effectiveness of the treatment. In this paper, we present a novel tool to extract motion features using single camera video recordings of infants. The Movidea software was developed to allow the operator to track the movement of end-effectors of infants in free moving conditions and extract movement features automatically. Movidea was used by different operators to analyze a set of video recordings and its performance was evaluated. The results showed that Movidea performance did not vary with the operator, and the tracking was also stable in home-video recordings. Even if the setup allowed for a two-dimensional analysis, most of the informative content of the movement was maintained. The reliability of the measures and features extracted, as well as the easiness of use, may boost the uptake of the proposed solution in clinical settings. Movidea overcomes the current limitation in the clinical practice in early detection of neurodevelopmental disorders by providing objective measures based on reliable data, and adds a new tool for the motor analysis of infants through unobtrusive technology.

**Keywords:** motion analysis; video signal processing; neurodevelopmental disorders; infant screening

## 1. Introduction

Early detection of neurodevelopmental disorders is of paramount importance. Indeed, providing timely interventions during infancy maximizes the outcomes of the long-term prognosis of affected children, capitalizing on the high neuroplasticity characterizing this period of life [1].

Motor skills shown during infancy have been found to be predictors of cognitive impairments arising in later developmental stages [2,3], thus indicating motor assessment as a valuable tool to early detect signs of neurodevelopmental disorders in infants.

Currently, in clinical practice, several tests are used to evaluate the motor performances of children at different ages. Nonetheless, such approaches suffer from major shortcomings. Some tests require the children to perform specific actions or to interact with objects [4,5], thus limiting their application to infants. Other tests rely on the subjective observation and rating of parents [6]. However, it should be noted that tests adopted depend on the subjective evaluation, rating, and experience of the examiner.

Another technique allowing the early detection of neuromotor diseases of infants is the Prechtl method of general movements (GMs) assessment [7]. GMs consist of complex movements in which all parts of the body participate. Typical general movements are characterized by complexity and

variation, whereas atypical general movements exhibit a limited repertoire of movement variants [8]. There is wide consensus that GMs are expression of the young developing brain, and their quality is an index of the integrity of the developing cortical network [8]. Their assessment according to Pretchl's method has been proven to predict cerebral palsy with a sensitivity greater than 91% and a specificity greater than 81% [9]. Moreover, GMs quality has also been associated with cognitive impairment, attention-deficit-hyperactivity disorder, and minor neurological dysfunction [10,11].

This method involves the qualitative evaluation by an expert observer of the features characterizing spontaneous general movements, recorded while the infant is in an awake calm state, lying in the supine position [12]. Even if GMs assessment is one of the most reliable methodologies for neurodevelopmental disorders detection, the need for a trained expert observer and the subjective and qualitative nature of the GMs assessment reduce the widespread and applicability of this assessment in daily clinical practice [13,14].

Technology-based automatic analysis of motor performances may represent a solution for providing low-cost objective evaluations. With this goal, different approaches have been proposed to track, quantify, and analyze the motor behavior in infants.

Wearable sensors such as accelerometers [15] and electromagnetic tracking systems [16] have been used to estimate the motion of the infants' limbs. These systems result in being too cumbersome to be applied to infants and require accurate calibration and positioning procedures.

Optical motion capture systems have also been proposed [17] to perform movement analysis of children's limbs. In [17], an optoelectronic system (6 cameras, 18 markers) was used to describe the movement of the infants. A set of metrics was computed on the basis of the extracted kinematic data, and the findings showed these metrics as being able to identify infants with spasticity correctly. Even if this approach ensures an accurate motion tracking and measurement, it requires devoted high-cost equipment and a time-consuming preparation process, making it not applicable outside of dedicated labs.

In [18], the kinematics of hand movements in infants was studied using video analysis to identify markers of neurodevelopmental disorders. Although the results showed that kinematics in infants with neurodevelopmental disorders present characteristics identifiable through video movement analysis of upper limbs, the applicability of the method was limited by the setup used. Two video cameras were needed to monitor a single limb, and a visual marker (i.e., wristband) was applied to the infants' wrist for analysis, affecting the conditions of the recorded infants.

Another approach is presented in [19], where a 3D camera was used to capture RGB and depth information from infants lying on their back, and an anatomical model was used to fit the data and reconstruct the movement. The study showed the applicability of this approach to GMs analysis, but its actual usage requires very high computational power, the storage of a large amount of data, and the manual intervention of a technical expert. These limitations limit the transferability of this approach to everyday clinical practice. A review of the currently available technology used to perform movement analysis in newborns for assessing GMs investigated the automatic analysis of video recordings [20]. The potential of this technique relies on the high availability of commercial video cameras and in the large amount of information recorded.

In the present study, we introduce a novel software (Movidea) that is based on semi-automatic video-based analysis of infants' motor performance. Movidea involves the tracking of infants' limbs using video recordings acquired by a single camera and the extraction of features for the description and evaluation of infants' motion during free movement conditions.

## 2. Materials and Methods

### *2.1. Video Database of the NIDA Network*

The Italian Network for early Detection of Autism spectrum disorder (ASD) (NIDA network) is the largest Italian cohort of infants at risk for AS. The NIDA network enrolls high risk infants (i.e., siblings

of children with a diagnosis of ASD, preterm newborns, and small for gestational age newborns) and low risk infants (i.e., siblings of typically developing children) after delivery with the aim of recording and assessing infant crying and spontaneous movements at 10 days, and 6, 12, 18, and 24 weeks of age. In addition, a comprehensive clinical evaluation of the infants/toddlers was performed at 6, 12, 18, 24, and 36 months. The study was carried out according to the standards for good ethical practice and the guidelines of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Istituto Superiore di Sanità (Approval Number: Pre 469/2016). Written informed consent from a parent/guardian of each participant was obtained.

The video recording of the infant's movements was generally performed at home while the child was lying on a bed, upon a green blanket provided by the NIDA network. The camera was placed 50 cm above the child, at chest height. The recording took place for at least 5 min with the aim of acquiring images of spontaneous movement of the full body of the child. To be analyzed with Movidea, each video recording was edited offline. A preliminary analysis of the videos showed that the high-quality video of all segments (i.e., without external interferences) did not exceed 3 min. Thus, we decided to save a 3 min video segment that represented the shorter high-quality frame for each recording. One author cut each video to ensure the same properties: 3 min length, infant in supine position, in a condition of well-being and spontaneous motor activity, without crying episodes. If videos showed more than 3 min of high quality frame, we decided to analyze the first high quality 3 min. Videoframes containing interferences by the operator and parents, as well as accidental movements of the camera, were excluded from the analysis.

For this study, 300 videos from the NIDA database were analyzed. A total of 90 infants were video recorded (mean gestational age at birth = 39.05 ± 1.35 weeks, mean body weight at birth 3300.98 ± 383.78 g, mean body length at birth = 50.27 ± 1.76 cm). Infant risk status, sex, and age at recording are reported in Table 1.

**Table 1.** Characteristics of infants video-recorded using a 2D camera.

| **Subjects** | | **Age of Recording** | | | | |
|---|---|---|---|---|---|---|
| Risk | Sex | 10 days | 6 weeks | 12 weeks | 18 weeks | 24 weeks |
| | | *n* | *n* | *n* | *n* | *n* |
| Low risk | M | 14 | 23 | 22 | 20 | 18 |
| | F | 8 | 15 | 16 | 9 | 11 |
| High risk | M | 13 | 14 | 16 | 16 | 13 |
| | F | 13 | 14 | 16 | 16 | 13 |

Infant risk status, sex, and age at recording using a 3D camera are reported in Table 2.

**Table 2.** Characteristics of infants video-recorded using a 3D camera.

| **Subject** | **Risk** | **Sex** | **Age of Recording** |
|---|---|---|---|
| 1 | Low risk | F | 12 weeks |
| 1 | Low risk | F | 18 weeks |
| 1 | Low risk | F | 24 weeks |
| 2 | Low risk | M | 12 weeks |
| 2 | Low risk | M | 24 weeks |

### *2.2. Movidea Software*

Movidea develops upon the arising need to identify early markers of neurodevelopmental disorders in infants, obtained through objective measures taken outside the clinical settings. In order to respond to this need, the software was designed to extract kinematic features of limbs from single-camera video recordings acquired in free movement conditions. The features were computed using two different approaches. On one hand, the trajectories covered by the infant's limbs during

the free movement were extracted using a semi-automatic limbs' tacking procedure. On the other hand, movement quantification was performed through image processing techniques applied to the video frames. The software was developed using MATLAB ver. R2017a and its standard tools. The Movidea software was implemented for and is owned by the Italian research governmental institution, Istituto Superiore di Sanità, and by the Ministry of Health that funded the NIDA Network project. The software was implemented exclusively for research purposes.

The overall workflow of the software is reported in Figure 1.

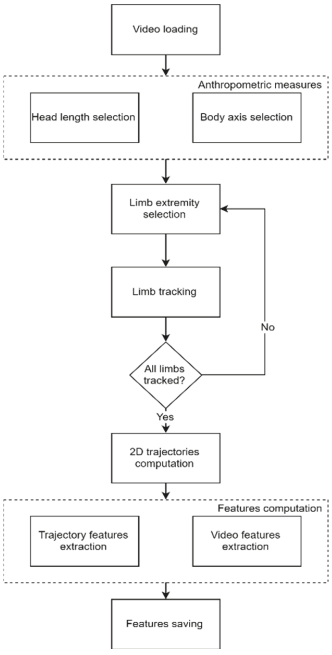


**Figure 1.** Movidea workflow.

The software was designed to allow the operators to go easily through the complete software workflow. A Graphical User Interface was developed to guide the software operator through each step. The operators were equipped with a user manual describing the software and all the interaction modalities, but no specific training was provided by technical experts. This aspect highlights the general usability of the software and easiness of operation deriving from the proposed approach.

### *2.3. Movement Tracking*

The absolute distance could not be measured using one camera setup, and thus the 2D tracked trajectories needed to be measured in pixels. Indeed, the relation between the pixel and the actual distance measure depended on several factors such as camera resolution and camera–subject distance, making this relation not constant outside the single video framework. Thus, using the pixel as the measurement unit did not allow for the comparison of the data among different videos.

To overcome this issue, the measure, in pixels, of the head length was used to normalize the data as anthropometric-related information suitable for allowing comparisons along time and subjects. The selection of the head length measure was the first step required by the software before proceeding with the tracking, and it was performed by manually setting the starting and the ending point of the line connecting the forehead and the chin of the infant in a video frame where both the points were clearly visible (Figure 2).


**Figure 2.** Head length line drawing. The red line connecting the forehead to the chin represents the head length measure taken by the operator.

Besides the head length, the operator was requested to select the central line of infant's body (symmetry line) as the perpendicular line running down the surface of the body passing from the midpoint of the clavicle-line to the midpoint of inferior margin of the pelvis (Figure 3). This operation allowed the operator to compute the body orientation in the image frame and, therefore, to represent the trajectories with standard orientation and to perform a final visual check of the data quality.


**Figure 3.** Body central line drawing. The red line connecting the clavicle-line mid-point to the inferior margin of the pelvis represents the body symmetry line taken by the operator.

Once the reference measures were taken, the limbs tracking can be performed. For each limb, the tracking required the operator first to identify the limb by selecting the central point of the end effector (i.e., hand, foot). The selected point was then tracked frame by frame using the Kanade–Lucas–Tomasi (KLT) algorithm [21]. To reduce the computational load and false positives, the algorithm was configured to search for the matching point in a squared area with a side size equal to 25% of the head length, centered in the coordinates of the point identified in the previous frame. In case the algorithm failed to locate the point in a frame, the operator could manually re-set the point to be tracked. If the tracked end effector was not visible in the frame (e.g., hidden by other body segments), the operator could skip the frame, avoiding producing invalid data.

The result of the tracking process for each limb was a $N \times 2$ matrix containing the coordinates of the end effector's reference point in the image for each of the $N$ frames of the video (Figure 4).


**Figure 4.** Trajectories represented here by lines of the four limbs tracked during an acquisition. Blue line = right hand; red line = left hand; yellow line = right foot; purple line = left foot; central green line = body symmetry line.

The trajectories were then normalized by the head length, and a linear interpolation was applied to compensate the missing values corresponding to the skipped frames. Indeed, if a limb was not tracked for a long time period, the interpolation may produce an artificial trend in the data and may compromise the informative content. For this reason, the data were not interpolated in case the limb presented more than five consecutive missing values. As the sampling rate of the analyzed videos was 12.5 Hz, the maximum time interval for the interpolation of missing data was equal to 400 ms.

The preprocessed trajectories were used for the computation of a set of movement features meaningful for the identification of pathological motion patterns [17]:

*Velocity and Acceleration*—The velocity was computed for each limb as the Euclidian distance of the reference point's location between two subsequent frames. The fast oscillations of the velocity profiles were then canceled through a third order low-pass Butterworth filter, with a cut-off frequency equal to the 95% of the Nyquist frequency. The acceleration of each limb was computed as the difference between two subsequent velocity samples. The mean velocity and mean acceleration of each limb was computed.

*Cross-correlation (CC)*—The zero-lag cross correlation between the velocity of each pair of limbs was computed as reported in [14], using the following equation:

$$CC_{v1v2} = \frac{\sigma_{v1v2}}{\sqrt{\sigma_{v1}^2 * \sigma_{v2}^2}} \quad (1)$$

where $CC_{v1v2}$ is the cross-correlation between the velocity $v1$ and the velocity $v2$, $\sigma_{v1v2}$ is the covariance of $v1$ and $v2$, $\sigma_{v1}^2$ is the variance of $v1$, and $\sigma_{v2}^2$ is the variance of $v2$.

CC is a measure of the synchronicity of the movements of the limbs, and it is a suitable marker of neurodevelopmental disorders in infants [17].

*Area differing from moving average ($A_{ma}$)*—For both the $x$ and $y$ components of the trajectory of each limb, the moving average was computed over the whole recording by using a window with a size of 30 samples according to the following equation:

$$\overline{x}_i = \frac{1}{k} \sum_{j=i-\frac{k}{2}}^{i+\frac{k}{2}} x_j \quad (2)$$

where $\overline{x}_i$ is the moving average computed at the $i$-th frame, $k$ is the window's size, and $x$ is the point position in the $j$-th frame.

The window size was chosen to average over 2 s, as reported in [17]. For each sample of the trajectory, the difference between the trajectory and the moving average was computed according to the following equation:

$$A_{max} = \sum_{i=\frac{k}{2}}^{l-\frac{k}{2}} \left| x_i - \overline{x}_i \right| \quad (3)$$

where $A_{max}$ is the area differing from the moving average of the $x$ component and $l$ is the total number of frames of the recording.

Moreover, the total $A_{ma}$ was calculated for the lower and the upper limbs as the sum of the area differing from the moving average of the two components of the two hands and the two feet, respectively. The $A_{ma}$ represents an index of the smoothness of the movements and it is a marker of neurodevelopmental disorders in infants [17].

*Periodicity (P)*—Periodicity is a parameter defined in [17] aimed at measuring the presence of repetitive movements in the motion of the limbs. To compute the periodicity, the recording was split into windows of 500 samples. In [17], the size of the window corresponded to one third of the total recording duration. To keep the computation coherent independently from the video length, the window's size was chosen to guarantee the same time span of 40 s used in [17]. For both the components of the movement of each limb, the mean of the trajectory was computed over each window, and the intersections of the trajectory with the mean were detected. The mean distance $\overline{d}$ and the standard deviation $\sigma_d$ between consecutive intersections were computed. Finally, the periodicity $P$ was computed by combining the parameters mentioned above, according to the following equation:

$$P = \frac{1}{\overline{d} + \sigma_d} \quad (4)$$

### 2.4. Image Processing

The image processing approach leverages on the movement quantification from the changes occurring in the image from one frame to the next one. To this goal, the first step of the processing was the creation of motion images where only the pixels changed in one frame with respect to the previous one due to the infant's movement were represented. In motion images, each pixel can assume only a value of 1 or 0, 1 (white) representing the occurrence of movement, and 0 (black) representing the absence of movement.

To obtain the motion images, the image of each frame was converted to black and white, and the difference with the black and white image of the previous frame was computed, resulting in a new image representing the changes occurring between the two frames. In order to account only for the changes related to the infant's movement, a 2D median filter was applied to $5 \times 5$ pixel areas to remove salt and pepper noise. The pixels overcoming a predefined threshold were then set to 1, and all the other pixels were set to 0. The threshold was chosen as the optimal value for reducing the noise due to change in the light conditions and presence of blurry images, avoiding at the same time the suppression of actual movements of the limbs. For removing the residual noise present on the images, a convolutional filter with a $3 \times 3$ equally weighted kernel was finally applied.

The motion images were used to compute several features related to the pathological conditions [22]:

*Quantity of motion (Q)*—is the number of pixels where the movement has occurred, divided by the total number of pixels in the image. The mean ($Q_{mean}$), the standard deviation ($Q_{sd}$), and the maximum value ($Q_{max}$) are computed [22].

*Centroid of motion (C)*—is a parameter representing the central point of the infant's movement in a given motion image. C is computed as the centroid of the cluster resulting from the application of a one-cluster $k$-means to the movement pixels of each motion image. The mean values $C_{xmean}$ and $C_{ymean}$ of C in $x$ and $y$ directions are computed over the recording together with the standard

deviations $C_{xsd}$ and $C_{ysd}$ [14]. The mean and the standard deviation of the velocity ($V_{mean}$, $V_{sd}$) and the acceleration ($A_{mean}$, $A_{sd}$) of the centroid are also computed.

### *2.5. Software Validation*

In order to verify the independence of measures extracted from the operator, a subset of 10 videos was analyzed through Movidea by two independent users, sharing the same instructions on how to operate the software.

The trajectories obtained by the scoring were compared between the two operators by computing the zero-lag correlation coefficient. Indeed, this approach allowed for a trend comparison rather than a comparison of the absolute position of the tracked point, which did not affect the final measures.

In addition, the consistency of the features extracted by the two operators was tested. To this scope, the intraclass correlation coefficient (ICC) [23] was computed using a two-way random single measure absolute agreement model [24]. The ICC was computed only for the features extracted from the trajectories, as the image processing features were automatically extracted and were independent of the operator intervention.

The tracking failure rate was computed as the percentage of the number of times the operator had to manually re-set the tracking point, with respect to the total number of frames. This score was computed on a sample of 300 analyzed video segments.

Another important issue to be verified involving assessing the methodology that was implemented in Movidea was the dimensionality of the information. The single camera setup resulted in a reduction of the three-dimensional motion of the limbs to a bidimensional space implying a reduction of information. Given these considerations, it is useful to quantify the information loss. For this purpose, we recorded five infants' videos using a 3D camera (RealSense D435, Intel, Santa Clara, CA, USA). Through the 3D camera, the RGB video and the depth information were recorded. The depth and RGB images were registered to obtain the 3D coordinates of the recorded points. The RGB videos were analyzed using Movidea, and the tracked trajectories were mapped in the new 3D space. The features previously described were computed on the 3D trajectories. The $z$-axis contribution was estimated on the features computed on the single axes (i.e., $A_{ma}$, $n_{int}$, $\overline{d}$, and $P$) as the percentage of the feature computed on $z$ with respect to the sum of the features computed on $x$, $y$, and $z$.

## 3. Results

Movidea was successfully used by non-technical operators to analyze over 300 video segments of infants, without major issues reported and without the intervention of a technical expert.

The mean correlation coefficients were computed between the trajectories obtained by the two operators for each video analyzed. The mean values of the correlation coefficients are reported in Table 3.

**Table 3.** Trajectories' correlation coefficients. For each axis of each limb, the mean ± SD of the correlation coefficients computed between the trajectories obtained by the two operators in each analyzed video is reported.

| Limb | Axis | Correlation Coefficient |
|---|---|---|
| Right Hand | $x$ | 0.991 ± 0.004 |
| | $y$ | 0.990 ± 0.005 |
| Left Hand | $x$ | 0.992 ± 0.003 |
| | $y$ | 0.980 ± 0.035 |
| Fight Foot | $x$ | 0.989 ± 0.005 |
| | $y$ | 0.966 ± 0.037 |
| Left Foot | $x$ | 0.973 ± 0.028 |
| | $y$ | 0.964 ± 0.034 |

The results show that the tracked trajectories were highly correlated and, thus, the tracking procedure was stable across different operators.

The ICC coefficients reported in Table 4 were higher than 0.75 for all the features, indicating an excellent degree of agreement between the measures taken from the two operators [25].

**Table 4.** Intraclass correlation coefficients (ICCs) for the features extracted from the tracked trajectories. The ICC coefficients were computed using the features extracted from a set of five videos analyzed by two operators.

| Feature | ICC |
|---|---|
| Mean velocity | 0.98 |
| Mean acceleration | 0.99 |
| Area from moving average | 0.97 |
| Cross-correlation coefficient | 0.96 |
| Intersections mean distance | 0.87 |
| Total number of intersections | 0.94 |
| Periodicity | 0.97 |

The results of the analysis of the third-dimension impact reported in Table 5 show that the information loss due to the dimensionality reduction was 36.7% on average with a maximum of 53%, highlighting that the two-dimensions features accounted for most of the informative content, but that the analysis may have taken advantage of a three-dimensional data acquisition setup easily obtainable thanks to the wide availability of mainstream commercial RGB and depth cameras, their encumbrance, and costs.

**Table 5.** Contribution of $z$-axis to the total. For each feature, the mean ± SD contribution of the $z$-axis to the feature value is reported.

| Feature | Name | $z$ Contribution (%) |
|---|---|---|
| $A_{marh}$ | Area from moving average right hand | 36.7 ± 3.4 |
| $A_{malh}$ | Area from moving average left hand | 41.6 ± 5.5 |
| $A_{marf}$ | Area from moving average right foot | 37.9 ± 4.4 |
| $A_{malf}$ | Area from moving average left foot | 35.7 ± 1.4 |
| $\overline{d}_{rh}$ | Intersections mean distance right hand | 16.8 ± 6.9 |
| $\overline{d}_{lh}$ | Intersections mean distance left hand | 11.3 ± 1.1 |
| $\overline{d}_{rf}$ | Intersections mean distance right foot | 16.5 ± 6.2 |
| $\overline{d}_{lh}$ | Intersections mean distance left foot | 18.0 ± 4.3 |
| $Tin_{rh}$ | Total number of intersections right hand | 44.0 ± 10.0 |
| $Tin_{lh}$ | Total number of intersections left hand | 53.9 ± 2.5 |
| $Tin_{rf}$ | Total number of intersections right foot | 45.9 ± 10.8 |
| $Tin_{lf}$ | Total number of intersections left foot | 43.4 ± 8.1 |
| $P_{rh}$ | Periodicity right hand | 46.2 ± 10.1 |
| $P_{lh}$ | Periodicity left hand | 52.4 ± 1.7 |
| $P_{rf}$ | Periodicity right foot | 49.1 ± 11.8 |
| $P_{lf}$ | Periodicity left foot | 47.2 ± 12.7 |

Finally, in Table 6, the mean percentage of the tracking failures with respect to the total number of frames is reported for each end-effector.

**Table 6.** Mean ± SD percentage of tracking failures. For each tracked limb, the percentage of frames in which the operator reset the tracking point is reported.

| End-Effector | Failure (%) |
|---|---|
| Right hand | 9.7 ± 6.7 |
| Left hand | 10.3 ± 6.7 |
| Right foot | 15.2 ± 9.3 |
| Left foot | 14.5 ± 9.2 |

## 4. Discussion

The goal of this paper was to evaluate if an automatic extraction of quantitative measures from video recordings could describe motor behaviors of infants. To this aim, we developed and tested a software implementing a semi-automatic analysis of movements in infants using single-camera video recordings. The software computes a set of features chosen according to their reported relevance in the literature and the occurrence of neurodevelopmental disorders. In particular, two different classes of features for the description of movement in infants were investigated: features extracted from the analysis of the trajectories of the limbs and features extracted from the analysis of movement images. The first class of features included the set of variables that in [17] were shown to be correlated with the occurrence of neurodevelopmental disorders. Such features relied on the extraction of infants' kinematics from the sequence of images recorded in the video, as well as on the computation of parameters able to describe such kinematics. The second class of features implemented the metrics identified as predictors of the occurrence of neurodevelopmental disorders in [14] and in [22]. Differently from the first class of features, these parameters did not rely on kinematic information but take advantage of the changes in the sequence of images to infer information on the infant's motion.

Movidea software is a valuable tool for several reasons. First, the performed analysis showed that the implemented approach was user-independent, even if the operator had to interact with the software in the data extraction phase. The tracked trajectories and the features extracted did no vary when different users operated the analysis. This aspect is of paramount importance to assure the homogeneity of the measures when multiple operators elaborate a large amount of data. Second, the low percentage of failures in the tracking process showed that the tracking strategy implemented in Movidea well fitted recordings in real-life settings, allowing wide spreading of the method. Third, the choice to use a single camera approach highly enhances the usability of Movidea. Indeed, the use of unobtrusive and off-the-shelf technology may boost the uptake of technological solutions to investigate early motor development in populations at risk. The longitudinal assessment of motor functioning in populations at risk for neurodevelopmental disorder is worth exploring further because it may be useful in detecting social disorder or other developmental disorders [26–28]. By extracting meaningful information and objective, reliable data through a light setup and an easy to use tool, Movidea overcomes the current limitation, resulting in it being effectively applicable in multicentric and large population studies.

The results presented, nonetheless, showed that some information was lost due to the dimensionality reduction. Even if this loss did not compromise the validity of the approach, the use of 3D information may have added value to the analysis. To this purpose, an alternative solution for the data acquisition using a 3D camera combining RGB and depth information was proposed.

Overall, the results showed that Movidea is a reliable tool for the description of infants' movements from 2D video recordings. This is a promising approach that raises attention to the automatic analysis of movement. Indeed, recent studies have proposed different tools for the analysis of video recordings of infants. For example, in [27], an explorative methodology for the pose estimation of joints of infants in video recordings was reported, whereas in [28], a platform was implemented for performing video recordings of infants and for extracting the velocity and the acceleration of the limbs. Nonetheless, these studies aimed at facilitating the visual inspection of the recordings for the identification of GMs. Movidea takes a step forward, producing a large set of features, both from

kinematics analysis and motion images, with the aim of moving from a qualitative visual analysis to a quantitative analysis of infants' movements.

**Author Contributions:** Conceptualization, M.B. and M.L.S.; software, W.B. and V.S.; investigation, W.B., F.F., L.G., and A.C.; formal analysis, W.B. and M.B.; writing—original draft preparation, W.B.; writing—review and editing, M.B., F.F., L.G., A.C., and M.L.S.; funding acquisition, M.L.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by Supported by the Italian Ministry of Health Network Project 'Italian Autism Spectrum Disorders Network: filling the gaps in the National Health System Care' (NET 2013- 02355263) and the MSCA-ITN-2018 - European Training Networks under grant agreement ID: 814302 (SAPIENS) "Shaping the social brain through early interactions".

**Conflicts of Interest:** The authors declare no conflict of interest.

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# Transcriptomic Analysis Reveals Abnormal Expression of Prion Disease Gene Pathway in Brains from Patients with Autism Spectrum Disorders

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Received: 26 February 2020; Accepted: 26 March 2020; Published: 29 March 2020

**Abstract:** The role of infections in the pathogenesis of autism spectrum disorder (ASD) is still controversial. In this study, we aimed to evaluate markers of infections and immune activation in ASD by performing a meta-analysis of publicly available whole-genome transcriptomic datasets of brain samples from autistic patients and otherwise normal people. Among the differentially expressed genes, no significant enrichment was observed for infectious diseases previously associated with ASD, including herpes simplex virus-1 (HSV-1), cytomegalovirus and Epstein–Barr virus in brain samples, nor was it found in peripheral blood from ASD patients. Interestingly, a significant number of genes belonging to the "prion diseases" pathway were found to be modulated in our ASD brain meta-analysis. Overall, our data do not support an association between infection and ASD. However, the data do provide support for the involvement of pathways related to other neurodegenerative diseases and give input to uncover novel pathogenetic mechanisms underlying ASD.

**Keywords:** autism; infection; prion; meta-analysis

## 1. Introduction

Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental disorders defined by significantly abnormal social interaction, impaired communication and language abilities, and a narrow pattern of interests [1]. It is estimated that the prevalence of ASD is 1%–2% in the general population with an average male-to-female ratio of 5:1 [2]. However, only about 10% of patients with a diagnosis of ASD have a defined etiology (so-called syndromic autism, secondary to Fragile X syndrome, neurofibromatosis and exposure to thalidomide) [3], while 90% of ASD cases are considered idiopathic.

Many authors have hypothesized a connection between genetic and epigenetic factors in ASD etiopathogenesis. In particular, infections have been suggested as a potential trigger of the disease [4–6]. In line with this, altered cellular immunity and an altered cytotoxic function of natural killer (NK) cells have been reported in ASD patients [7–9]. It has also been shown that fungal mycotoxins, such

as deoxynivalenol in urine and Ochratoxin A in serum, are increased in autistic children [10,11]. Finally, expression of immune response genes has been described in cortical tissues from older ASD subjects [12,13].

In the present study, we investigated the expression levels of transcriptional markers of infections and immune activation in brain and blood samples from autistic patients by performing a meta-analysis of publicly available whole-genome expression datasets. The analysis of the data suggests common transcriptional features between ASD and prion-related diseases but does not support the role of infectious disease in the etiopathogenesis of ASD.

## 2. Materials and Methods

### *2.1. Data Collection and Metanalysis*

The NCBI (National Center for Biotechnology Information) Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) was used to identify microarray datasets comparing the transcriptomic profiles of healthy donors and ASD patients. The GEO database was manually searched using the terms "autism" and "autistic disorder". The collected datasets were further selected if they met the following inclusion criteria: (a) whole-genome transcriptomic profiling; (b) brain or blood samples; (c) consisted of one cohort of ASD patients and another cohort of healthy people; and (d) species of origin was "*Homo sapiens*". Finally, five datasets were included in the meta-analysis of brain samples: GSE28521, GSE38322, GSE62098, GSE64018 and GSE102741, while three datasets were used for the meta-analysis of blood samples: GSE6575, GSE42133 and GSE18123. When a dataset included more than one tissue type, data from each tissue type were processed as a separate dataset. The datasets were uploaded to NetworkAnalyst 3.0 software (Ste. Anne de Bellevue, Quebec, Canada). Data were auto-scaled, and an integrity check was performed prior to the meta-analysis stage. Batch effects were corrected using the "ComBat" function. A random effects model of effect size (ES) measure was used to integrate gene expression patterns from the three datasets. The random effects model presumes that different studies present substantial diversity and evaluates between-study variance as well as within-study sampling error. Genes with a False Discovery Rate (FDR) < 0.05 were identified as differentially expressed genes (DEGs) and selected for further analysis. The characteristics of the samples in the datasets used are described in Table 1.

**Table 1.** Characteristics of the datasets used in the meta-analyses.

| Dataset ID | Tissue | Samples | Platform | Reference |
|---|---|---|---|---|
| GSE28521 | Temporal cortex | $n$ = 13 ASD<br>$n$ = 13 HD * | Illumina HumanRef-8 v3.0 Expression BeadChip | [14] |
| | Frontal cortex | $n$ = 16 ASD<br>$n$ = 16 HD * | | |
| | Cerebellum | $n$ = 10 ASD<br>$n$ = 11 HD * | | |
| GSE38322 | Occipital cortex (BA19) | $n$ = 6 ASD<br>$n$ = 4 HD * | Illumina HumanHT-12 V4.0 Expression BeadChip | [15]<br>[16] |
| | Cerebellum | $n$ = 8 ASD<br>$n$ = 8 HD * | | |
| GSE62098 | Corpus callosum | $n$ = 6 ASD<br>$n$ = 6 HD * | Illumina HiSeq 2000 (*Homo sapiens*) | [17] |
| GSE64018 | Superior temporal gyrus | $n$ = 12 ASD<br>$n$ = 12 HD * | Illumina HiSeq 2000 (*Homo sapiens*) | [18] |
| GSE102741 | Dorsolateral prefrontal cortex | $n$ = 13 ASD<br>$n$ = 39 HD * | Illumina HiSeq 2000 (*Homo sapiens*) | [19] |

**Table 1.** *Cont.*

| Dataset ID | Tissue | Samples | Platform | Reference |
|---|---|---|---|---|
| GSE102741 | Dorsolateral prefrontal cortex | $n$ = 13 ASD<br>$n$ = 39 HD * | Illumina HiSeq 2000 (*Homo sapiens*) | [19] |
| GSE6575 | Whole blood | $n$ = 35 ASD<br>$n$ = 12 HD * | Affymetrix Human Genome U133 Plus 2.0 Array | [20] |
| GSE42133 | Leukocytes | $n$ = 91 ASD<br>$n$ = 56 HD * | Illumina HumanHT-12 V4.0 Expression BeadChip | [21]<br>[22] |
| GSE18123 | Whole blood | $n$ = 31 ASD<br>$n$ = 33 HD * | Affymetrix Human Genome U133 Plus 2.0 Array | [23] |

* HD: Healthy donors.

### 2.2. Pathway Selection and Gene Intersection

Pathway enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/) implemented in the Enrichr (http://amp.pharm.mssm.edu/Enrichr) web-based utility [24]. Higher-level biological functions are represented by networks of molecular interactions, reactions and relations that are integrated in the pathways from the KEGG database. KEGG integrates the current knowledge on molecular interaction networks and uses a knowledge-based approach for network prediction that aims to predict, given a complete set of genes in the genome, the protein interaction networks that are responsible for various cellular processes [25]. Enrichr computes the $p$ value using the Fisher exact test. The adjusted $p$ value is calculated using the Benjamini–Hochberg method for correction for multiple hypotheses testing. The z-score is computed using a modification to the Fisher exact test and assesses the deviation from the expected rank. Finally, the combined score is calculated using the $p$ value and the z-score (Combined Score = $\ln(p \text{ value}) \times$ z-score).

### 2.3. Machine Learning Prediction and Network Construction

The webtool "ASD Genome-wide predictions of autism-associated genes" was used to evaluate the probability value of association between the selected gene and ASD. This webtool is based on a machine learning approach that, using a Bayesian method, allows the user to predict the role of candidate genes [26]. Briefly, Krishnan et al. developed an evidence-weighted, network-based machine-learning method that uses this brain-specific network to systematically discover new candidate ASD risk genes across the genome. The brain-specific network was constructed using a Bayesian method that extracts and integrates brain-specific functional signals from a gene-interaction network model containing predicted functional relationships for all pairs within 25,825 genes in the human genome. In order to produce a comprehensive, robust, genome-wide ranked list of autism candidate genes, Krishnan et al. first curated 594 genes linked with autism from publicly available databases and based on the strength of evidence of association with ASD. Next, an evidence-weighted support vector machine classifier, using the connectivity of genes to all the genes in the human brain-specific network, was employed to identify novel ASD candidates, defined as those genes whose interaction features in the network most closely resemble those of known ASD-related genes [26].

### 2.4. Statistical Analysis

For the meta-analysis, a random-effect model of effect size measure was used to integrate gene expression patterns from the selected datasets. Genes with an adjusted $p$ value (FDR, q-value) $< 0.05$ were identified as DEGs and selected for further analysis. Pathway enrichment analysis was performed using the online server Enrichr (http://amp.pharm.mssm.edu/Enrichr) [24]. For all the analyses, an adjusted $p$ value $\leq 0.05$ was considered as the statistical significance threshold.

## 3. Results

### *3.1. Identification of an ASD Brain Transcriptomic Profile*

Five GEO whole-genome transcriptomic datasets were identified (see Table 1) and used in the following analysis. These datasets included 84 brain samples from ASD patients ($n$ = 55 unique patients) and 109 brain samples from otherwise normal people ($n$ = 81 unique subjects). The meta-analysis identified 516 DEGs: 218 upregulated and 298 downregulated. The most enriched pathways were represented by "Synaptic vesicle cycle", "Huntington's disease" and "Sphingolipid signaling pathway" (Table 2).

**Table 2.** Top 10 enriched KEGG pathways in brain samples from ASD patients.

| Term | *p* Value | Adj. *p*-Value | Odds Ratio | Combined Score |
|---|---|---|---|---|
| Synaptic vesicle cycle | 8.95E-04 | 0.030642 | 3.975353 | 27.90006 |
| Huntington's disease | 5.06E-04 | 0.031192 | 2.811584 | 21.33503 |
| Sphingolipid signaling pathway | 0.001034 | 0.031855 | 3.257117 | 22.38962 |
| Thyroid hormone signaling pathway | 8.49E-04 | 0.03269 | 3.341353 | 23.62788 |
| Parkinson's disease | 3.20E-04 | 0.032842 | 3.275467 | 26.35943 |
| Gap junction | 4.43E-04 | 0.034103 | 3.964059 | 30.61119 |
| VEGF signaling pathway | 7.81E-04 | 0.034369 | 4.598607 | 32.90204 |
| Prion diseases | 2.46E-04 | 0.037893 | 6.644518 | 55.21555 |
| Valine, leucine and isoleucine degradation | 0.001402 | 0.03926 | 4.844961 | 31.83014 |
| Lysine degradation | 7.81E-04 | 0.040097 | 4.598607 | 32.90204 |

Figure 1 shows the results from the enrichment analysis for infectious-related pathways enlisted in the KEGG database. No significant enrichment was observed among the DEGs with the exception of the "prion diseases" pathway (q = 0.038) (Figures 1 and 2; Supplementary File 1). In particular, in the "prion diseases" pathway, our analysis identified significantly higher levels of Complement Component 1, q Subcomponent, B Chain (C1QB), Heat Shock Protein Family A Member 5 (HSPA5), Proto-Oncogene Tyrosine-Protein kinase Fyn (FYN), Laminin Subunit Gamma 1 (LAMC1) and ETS Like-1 Protein (ELK1) and significantly lower levels of Mitogen-Activated Protein Kinase 1 (MAP2K1) (Figure 2).

We next wanted to evaluate the enrichment of immune-related processes among the ASD brain DEGs. As shown in Figure 3, only the "Sphingolipid signaling pathway" was significantly enriched, encompassing four downregulated DEGs (MAP2K1, Protein Kinase C Beta (PRKCB), Sphingosine Kinase 2 (SPHK2), Ras-Related C3 Botulinum Toxin Substrate 3 (RAC3)) and six upregulated DEGs (G Protein Subunit Alpha I3 (GNAI3), Sphingosine-1-phosphate receptor 1 (S1PR1), FYN, Rapidly Accelerated Fibrosarcoma 1 (RAF1), TNF Receptor Superfamily Member 1A (TNFRSF1A), G Protein Subunit Alpha I2 (GNAI2)).

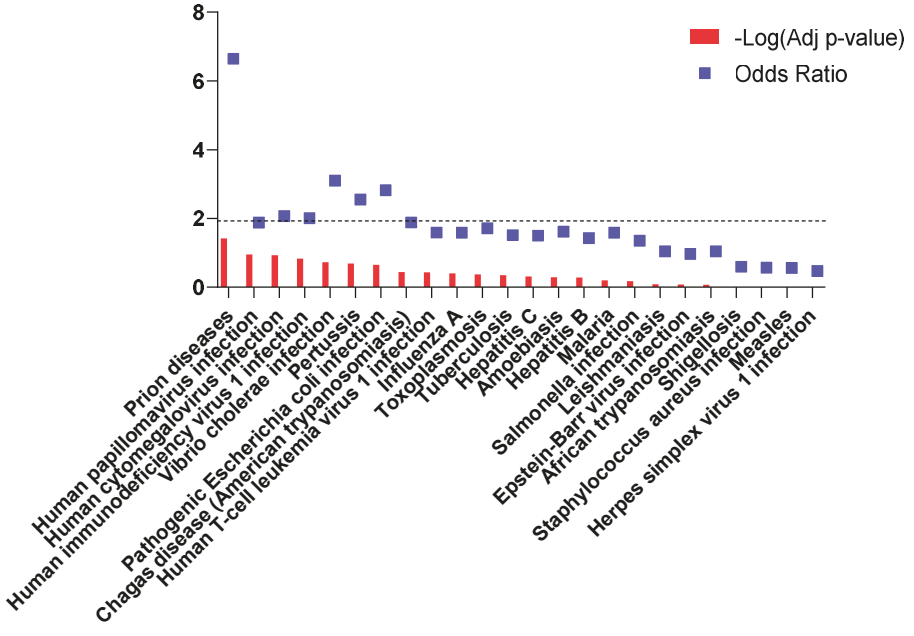


**Figure 1.** Infection-related pathways enriched in brain samples from ASD patients. Dotted line indicates the threshold of significance.

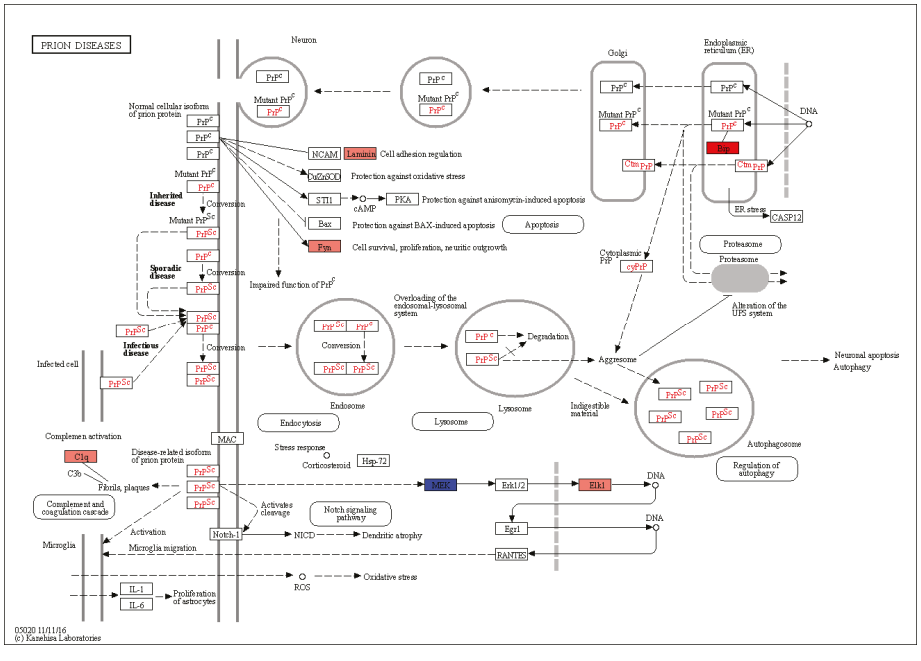


**Figure 2.** "Prion diseases pathway" from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database with genes significantly modulated in brain samples from ASD patients that have been color-coded from blue (downregulated) to red (upregulated).

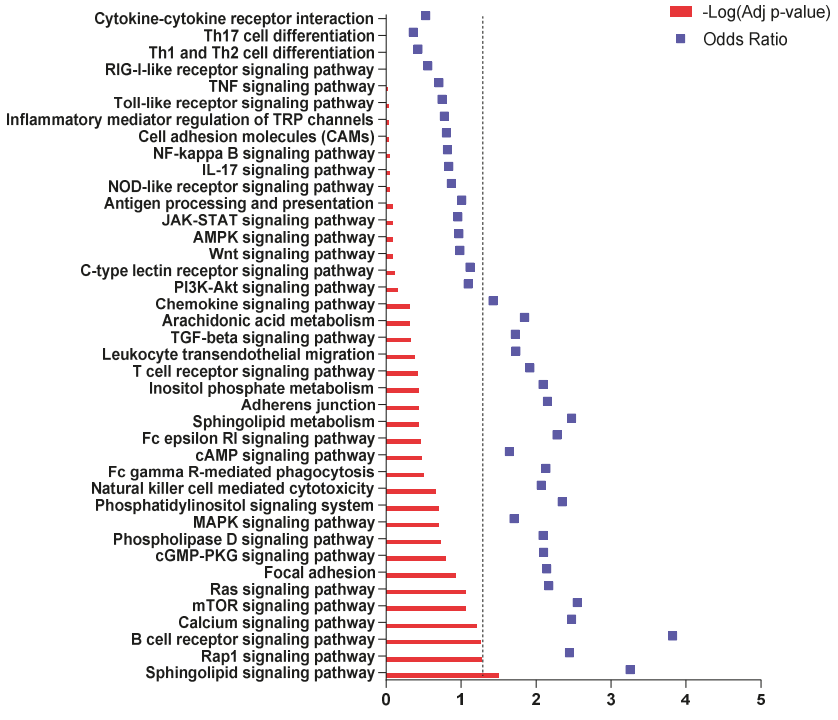


**Figure 3.** Immune-related pathways enriched in brain samples from ASD patients. Dotted line indicates the threshold of significance.

### *3.2. Machine Learning Prediction*

The brain autism DEGs belonging to the "prion diseases" pathway from the Kyoto Encyclopedia of Genes and Genomes (KEGG) were investigated for their potential role in ASD using a network machine learning approach implemented in the "ASD Genome-wide predictions of autism-associated genes" web-tool (http://asd.princeton.edu/). The network constructed using the brain ASD DEGs belonging to the "prion diseases" pathway is presented as Figure 4. Among the input genes, the only one significantly associated with ASD is FYN, with an estimated probability of 0.665 and a q-value = 0.0256. Table 3 shows the genes mostly interacting with the input genes, ordered by the edge score. The prioritization and prediction of ranking is based on the network-based approach developed by Krishnan et al. [26]. Among the top-ranking ASD genes associated with the DEGs belonging to the "prion diseases" pathway, Mesencephalic Astrocyte Derived Neurotrophic Factor (MANF), Heat Shock Protein 90 Beta Family Member 1 (HSP90B1) and Mitogen-Activated Protein Kinase 1 (MAPK1) showed edge scores of 0.791, 0.79 and 0.789 with HSPA5, HSPA5 and MAP2K1, respectively (Table 3). The top-ranking ASD gene interacting with the DEGs belonging to the "prion diseases" pathway is Ataxin 1 (ATXN1), with a rank position of 5, a probability value of association with ASD of 0.808 and a q-value = 0.0186. ATXN1 was the most connected gene to FYN (edge score 0.705) (Table 4). None of the predicted top 10 genes are present in the Genome-Wide Association Study (GWAS) Catalog 2019.

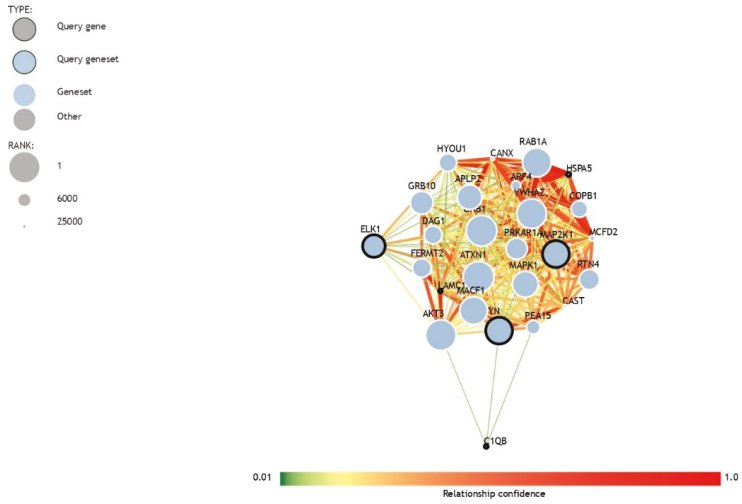


**Figure 4.** Network constructed using the differentially expressed genes in the ASD brain belonging to the "prion diseases" pathway using a minimum confidence score of 0.04 and a maximum of 20 interacting genes.

**Table 3.** Top 10 genes interacting with ASD brain DEGs belonging to the "prion diseases" pathway.

| Query Gene | Gene | Gene Description | Edge Score |
|---|---|---|---|
| HSPA5 | MANF | mesencephalic astrocyte-derived neurotrophic factor | 0.791 |
| HSPA5 | HSP90B1 | heat shock protein 90kDa beta (Grp94), member 1 | 0.79 |
| MAP2K1 | MAPK1 | mitogen-activated protein kinase 1 | 0.789 |
| HSPA5 | RAB1A | RAB1A, member RAS oncogene family | 0.761 |
| MAP2K1 | PGK1 | phosphoglycerate kinase 1 | 0.728 |
| MAP2K1 | YWHAZ | tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide | 0.706 |
| FYN | ATXN1 | ataxin 1 | 0.705 |
| HSPA5 | ARF4 | ADP-ribosylation factor 4 | 0.702 |
| HSPA5 | HERPUD1 | homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1 | 0.69 |
| LAMC1 | AKT3 | v-akt murine thymoma viral oncogene homolog 3 | 0.669 |

**Table 4.** Top 10 ranking ASD genes interacting with brain DEGs belonging to the "prion diseases" pathway.

| Gene | Description | Avg. Edge Score to Query | Rank | Probability of ASD Association | *p*-Value | q-Value |
|---|---|---|---|---|---|---|
| ATXN1 | ataxin 1 | 0.216 | 5 | 0.828 | 0.002 | 0.0186 |
| GNB1 | guanine nucleotide binding protein (G protein), beta polypeptide 1 | 0.231 | 24 | 0.811 | 0.001 | 0.0113 |
| AKT3 | v-akt murine thymoma viral oncogene homolog 3 | 0.205 | 75 | 0.722 | 0.001 | 0.0113 |
| YWHAB | tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide | 0.176 | 107 | 0.71 | 0.006 | 0.0438 |
| YWHAZ | tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide | 0.212 | 269 | 0.697 | 0.08 | 0.3199 |
| RAB1A | RAB1A, member RAS oncogene family | 0.257 | 453 | 0.696 | 0.066 | 0.2786 |
| MACF1 | microtubule-actin crosslinking factor 1 | 0.205 | 715 | 0.667 | 0.005 | 0.0381 |
| PPIB | peptidylprolyl isomerase B (cyclophilin B) | 0.152 | 753 | 0.666 | 0.221 | 0.6426 |
| BHLHE40 | basic helix-loop-helix family, member e40 | 0.187 | 1054 | 0.663 | 0.149 | 0.4976 |
| MAPK1 | mitogen-activated protein kinase 1 | 0.234 | 1128 | 0.661 | 0.133 | 0.4605 |

### *3.3. Identification of an ASD Blood Transcriptomic Profile*

Three GEO whole-genome transcriptomic datasets, GSE6575, GSE42133 and GSE18123, were identified, as indicated in Table 1, for the following analysis. These datasets included blood samples from 157 ASD patients and blood samples from 101 otherwise normal people. The meta-analysis identified only 24 DEGs: 19 upregulated and 5 downregulated. As shown in Table 5, no significant enrichment for any KEGG pathway was detected (Table 5).

**Table 5.** Top 10 enriched KEGG pathways in blood from ASD patients.

| Term | *p*-Value | Adjusted *p*-Value | Odds Ratio | Combined Score |
|---|---|---|---|---|
| Autophagy | 0.01023 | 0.450115 | 13.02083 | 59.6672 |
| Osteoclast differentiation | 0.010077 | 0.517306 | 13.12336 | 60.33413 |
| cGMP-PKG signaling pathway | 0.016766 | 0.573785 | 10.04016 | 41.04796 |
| Tuberculosis | 0.019321 | 0.595073 | 9.310987 | 36.74661 |
| Oocyte meiosis | 0.009775 | 0.60217 | 13.33333 | 61.70503 |
| Cellular senescence | 0.015641 | 0.602172 | 10.41667 | 43.31114 |
| AMPK signaling pathway | 0.009039 | 0.695998 | 13.88889 | 65.36409 |
| Thermogenesis | 0.031025 | 0.73505 | 7.215007 | 25.05748 |
| Regulation of actin cytoskeleton | 0.026947 | 0.754518 | 7.788162 | 28.14549 |
| Insulin resistance | 0.007379 | 0.757574 | 15.4321 | 75.75804 |

## 4. Discussion

According to the current DSM-5 criteria, two requirements are needed to obtain an ASD diagnosis: (1) persistent deficits in social communication and social interaction across multiple contexts, and (2) restricted, repetitive patterns of behavior, interests or activities [1]. Although ASD has a complex multifactorial etiology, twin studies have proven a strong genetic contribution, with a concordance rate of autistic disorders in monozygotic twins of 70%–90% and in dizygotic twins of 30% [27,28].

However, the complexity of the disease requires omics approaches to integrate and extrapolate more information. Genome-wide association studies, candidate gene studies and microarray experiments of differential gene expression have been largely used in autism. These studies produce extensive and information-rich data that represent a snapshot of all genetic and/or molecular events occurring in a diseased cell at one particular point in time and can be used to generate hypotheses. The use of whole-genome expression databases has been largely exploited by our group and others [29–33] for the characterization of the etiopathogenesis of a variety of diseases (e.g., autoimmune diseases [34–42] and cancer [36,43,44]) and has allowed researchers to characterize pathogenic pathways [45–48] and potential novel therapeutic targets [49–57].

Many authors have suggested that the role of infection during pregnancy or in the first phases of life could trigger the immune system to alter normal neurodevelopment, causing neuronal damage [8,58,59]. In particular, the role of the Herpesviridae family has been largely investigated. For instance, cytomegalovirus (CMV) can directly damage key structures in the developing brain when contracted during pregnancy [60], and indeed, in vitro studies have shown that CMV infection can inhibit neuronal differentiation and induce apoptosis in neural precursor cells [61,62]. Also, other infectious diseases such as influenza A [58], toxoplasmosis [63,64] and measles [6,65] are suspected to be related to ASD.

However, the role of infections in the pathogenesis of autism is still highly debated. The levels of D-arabinitol, a marker of candidiasis fungal infection, as well as of a phenylalanine metabolite of *Clostridia* species, the 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, are increased in the urine of autistic children [66,67]. Accumulating evidence also suggests that latent chronic toxoplasmosis plays a role in the triggering and development of many psychiatric and neurological disorders, including ASD [68]. On the other hand, other studies have not shown a significant prevalence of infections in ASD [5,69–71]. The aim of our analysis was to evaluate, by performing a meta-analysis of available

whole-genome transcriptomic datasets, whether infection alone or infection and immune activation processes could be detected in the brains or peripheral blood of autistic patients. To our knowledge, this is the largest meta-analysis of both ASD brain samples and leukocytes to date.

In our study, no significant enrichment for infection-related pathways, including Epstein-Barr virus (EBV), CMV, HSV-1, measles, influenza A and toxoplasmosis, was found among the DEGs identified in the meta-analyses. On the other hand, a significant enrichment of the "prion diseases" pathway was observed. However, it should be pointed out that, with the present data, it is currently not possible to identify ASD as a prion-related disease, but it is possible to describe common biomolecular pathways underlying ASD pathogenesis. Indeed, prion infection is known to affect microglial sensing and homeostasis ability and to reduce microglial phagocytosis of aberrant proteins, including PrPsc (scrapie isoform of the prion protein) and apoptotic debris or cells, despite production of proinflammatory mediators. Furthermore, the effects of PrPsc on microglia appear to be mediated by Toll-like Receptors (TLRs) in a Src-like kinase-dependent manner (reviewed in [72]). So, it may not be surprising to find that prion pathways are modified in the brains of ASD patients, as it may reflect prior inflammatory processes, having modified microglia.

In the present paper, we have combined transcriptomic meta-analysis, pathway enrichment and machine learning prediction in order to prioritize genes of interest with potential pivotal pathogenetic effects in autism. Computational methods have been largely used to investigate the etiopathogenesis of polygenic and idiopathic disorders. Functional interaction networks that integrate gene interaction data can be exploited to identify which genes are most strongly implicated in a disorder. Given a list of genes that are altered in a disease, we can apply methods to identify genes that are near the input genes within a functional interaction network that rely on the connections among genes in a functional interaction network. The major limitation of this kind of approach is that it relies on the methods of selection by which functional terms are included in the network-based prediction. Hence, the better tailored this set of genes is to the disease of interest, the higher reliability we have in the final predictions. The use of the machine learning prediction tool developed by Krishnan and colleagues [26] allows us to evaluate the probability value of association between the selected gene and ASD in the context of the human brain-specific network. With this approach, we likely arrive at a robust set of candidates that are relatively unbiased by previously published works. The final output of this strategy, i.e., a ranked list of candidate genes, is easy to interpret and provides a limited set of hypotheses to test in further investigations. However, while we cannot definitively identify the causal gene or genes, it does provide a much-reduced set of candidates to investigate. In particular, a role for tyrosine kinase Fyn is proposed. Fyn has been described as expressed in the mouse hippocampus, amygdala and cerebellum [73,74]. Mutations of Fyn in mice lead to alteration in the architecture of the hippocampus [75] with consequent impairment in learning and in the amygdala long-term plasticity [73]. Fyn regulates the focal adhesion kinase (FAK), which is required for normal neuronal development [73,76].

In our analysis, Fyn was strongly correlated with ATXN1, a DNA-binding protein that forms a transcriptional repressor complex with capicua (CIC). It has been previously described that the deletion in chromosome 6p22.3-p24.3, which harbors ATXN1, is associated with developmental delay and ASD [77,78]. Moreover, alteration of the ATXN1-CIC complex determines a spectrum of neurobehavioral phenotypes, including intellectual disability, attention deficit/hyperactivity disorder (ADHD) and autism spectrum disorder [79].

Finally, we need to address some important limitations to our study. First, the number of available gene expression datasets of brain samples derived from ASD patients is limited, and the number of samples included in each dataset is often negligible. Second, the meta-analysis here performed encompasses different brain regions (temporal, occipital and frontal cortex, as well as corpus callosum and cerebellum). These facts undermine the statistical power of the differential expression analysis and impede patients' stratification, in terms of clinical phenotype, which is advisable given the heterogeneity of ASD. It is likely that different subgroups of patients may have peculiar brain transcriptomic patterns. Moreover, gene expression analysis is not enough to determine whether particular biological processes

are activated or not, limiting the reliability of the conclusions that can be drawn. Hence, more population- and molecular-based studies are warranted to confirm or negate hypotheses.

Characterizing molecular pathways underlying ASD represents a crucial step for personalized medicine where comprehensive phenotyping of individual patients could be available, providing novel tailored treatment options. The data from this study suggest that infections may not necessarily be responsible for ASD development. However, since some genes involved in the infectious processes can interact with other key genes in autism, infections may likely act as co-factors, possibly causing worse clinical presentations. Future studies are necessary to validate these findings and prove if these genes can be used as biomarkers or even as eventual therapeutic targets. Finally, we have to point out that the present analysis cannot evaluate the potential role of infections in the prenatal period or contracted in the early stage of life.

## 5. Conclusions

In this paper, we investigated the relationship between infections and autism, proving that they should not be considered as etiological factors but probably as co-factors. We analyzed the gene expression profiles of brain and blood from autistic patients and compared them with the genes involved in the most frequent infectious diseases associated with pregnancy and suspected to be related to ASD. Our analysis does not show any statistically significant associations between ASD and previously studied infectious agents. However, it does show a statistical association between prion disease and autism. Finally, based on a Bayesian machine learning approach, we predicted that new genes may be associated with ASD and possibly, after validation, used as markers or therapeutic targets.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-3425/10/4/200/s1: File 1: Prion diseases pathway.

**Author Contributions:** Conceptualization, S.D.L., P.F. and E.C.; Data curation, M.C.P. and M.S.B.; Formal analysis, S.D.L. and P.F.; Funding acquisition, G.B. and V.B.; Supervision, F.N.; Visualization, S.D.L.; Writing—original draft, S.D.L., M.C.P., M.S.B. and E.C.; Writing—review & editing, G.B., K.M., V.B., P.F., R.B. and F.N. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported by current research funds 2019 of IRCCS "NEUROMED—Mediterranean Neurological Institute", Pozzilli, Italy.

**Acknowledgments:** Not applicable.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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# A Two-Stage Screening Approach with I-TC and Q-CHAT to Identify Toddlers at Risk for Autism Spectrum Disorder within the Italian Public Health System

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Received: 4 March 2020; Accepted: 21 March 2020; Published: 23 March 2020

**Abstract:** Standardized screening programs ensure that children are monitored for early signs of autism spectrum disorder (ASD) in order to promote earlier diagnosis and intervention. The aim of this study is to identify early signs of atypical development consistent with ASD or other developmental disorders in a population of 224 low-risk toddlers through a two-stage screening approach applied at 12 and 18 months of age. We adopted two screening tools combined: 1. the Communication and Symbolic Behavior Scales Developmental Profile (CSBS DP) Infant–Toddler Checklist (I-TC) and 2. The Quantitative Checklist for Autism in Toddlers (Q-CHAT). We assessed their sensitivity and specificity related to the diagnostic outcome at 36 months. The results showed that autistic signs can be detected as early as the first year even through a few questions extrapolated from both screeners and that our model could be used as a screening procedure in the Italian public health system.

**Keywords:** autism spectrum disorder; screening; early detection

## 1. Introduction

Autism spectrum disorder (ASD) is a heterogeneous complex of neurodevelopmental disorders distinguished by impairments in social communication, reciprocal interaction and repetitive pattern of behaviors and interests, as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) [1].

Clear evidence exists that early detection and early intervention can lead to a better prognosis [2–4]. According to the latest revision of the American Academy of Pediatrics (AAP) guidelines about promoting optimal development in infants and young children, the early identification of developmental disorders should be conducted through developmental surveillance and periodic screening at each pediatric health visit [5]. The AAP recommends that specific screeners for ASD should be administered to all children at their 18- and 24-month visits because screening tests enhance the accuracy of the developmental surveillance process [6]. On the other hand, many prospective studies investigating siblings of children with ASD, a high-risk population for ASD, showed that early signs of ASD can be identified as early as 12 months of age [7,8]. However, screenings conducted too early may not be able

to distinguish ASD from other developmental disorders, which correspond to the majority of false positive cases—or even from typical development [9].

Family and population studies have supplied evidence of a broader autism phenotype (BAP) referring to the presence of subclinical autistic traits in ASD-patient's relatives and in the general population, such as social-communication deficits and rigidity of personality and behaviors not severe enough to deserve a diagnosis of ASD [10–13]. It remains unclear whether, in early development, mild social communication deficits and personality rigidity are part of the BAP or they represent early signs of ASD because only a few studies have investigated BAP features in infancy and toddlerhood [14].

Despite the increase of developmental screening tools, it is likely that no single screening test is appropriate for all children at all ages [15]. Repeated and regular screenings may be more effective than a single screening to differentiate properly the early signs of ASD from other developmental conditions [9]. This statement is supported by a recent review containing six studies conducted in Europe on screening procedures and strategies, which suggest that an ASD population screening is more efficacious if it adopts a multi-stage approach and if it combines different screening tools in order to cover a wider range of age and severity of symptoms, thus minimizing the number of false negatives [16].

The aim of our study was to identify early signs of atypical development consistent with autism spectrum disorder (ASD) and broader autism phenotype (BAP) conditions in a population of low-risk toddlers through a two-stage screening approach. We combined two screening instruments for ASD that are not commonly used in the Italian context: 1. the Communication and Symbolic Behavior Scales Developmental Profile (CSBS DP) Infant–Toddler Checklist (I-TC) [17–19] and 2. the Quantitative Checklist for Autism in Toddlers (Q-CHAT) [20]. The two instruments were used in a two-stage screening approach at 12 and 18 months of age. Then, we followed the screen positive cases through consecutive evaluations of cognitive, language, motor and social skills until the final diagnostic outcome at 36 months of age. We chose the Q-CHAT questionnaire as a general assessment of autistic traits because it better explores the quantitative differences between ASD and general population; while the I-TC, originally developed for early detection of language delay, was chosen because of its emphasis on pre-linguistic communication and some social components that are key features of early ASD, including gestures and shared attention. Finally, we tried to identify from both screeners the items most sensitive to predict an ASD diagnosis to help clinicians in the referral process for a full diagnostic evaluation.

## 2. Materials and Methods

### *2.1. Study Design*

We report data from the administration of two short screening questionnaires: 1. the Quantitative Checklist for Autism in Toddlers (Q-CHAT) and 2. One measure of the Communication and Symbolic Behavior Scales Developmental Profile (CSBS DP), the Infant–Toddler Checklist (I-TC). These screeners were administered to an unselected population of toddlers. The screening protocol required the questionnaires to be administered personally to the parents by a child psychologist at 12 months and repeated at 18 months of age, regardless of the result of the first screening. The questionnaires were administered at 12 months in specialized public health vaccination centers where children received mandatory vaccinations, because, in Italy, vaccinations at 12 months are mandatory, while at 18 months the same psychologist administered the screeners by telephone. All parents agreed to participate in the study on a voluntary basis and provided informed consent. The study was approved by the Technical Scientific Committee of the Institute for Maternal and Child Health-IRCCS "Burlo Garofolo" in Trieste, Italy (Prot. CE/V-151).

Children who screened positive in both questionnaires at 12 months, and only in one of them at 18 months, were evaluated by a child neuropsychiatrist expert in autism who confirmed the ASD risk and recruited to participate in a longitudinal prospective study involving diagnostic evaluation

every 6 months (at 12-18-24-30-36 months) from the time of recruitment until 36 months of age. The diagnostic assessment was based on the clinical judgement and standardized tests' results for cognitive, language, motor and social domains. In case of diagnostic concerns, children were referred for early intervention. Families received diagnostic feedback at each follow-up visit. Moreover, the child's pediatrician received a letter describing the study prior to the beginning of the study, as well as screening and diagnostic evaluation reports. Data regarding the follow-up evaluations and, consequently, the description of the developmental trajectories will be described in a forthcoming publication, given that the focus of the current publication is on early detection of ASD.

The flowchart in Figure 1 describes the whole design of the study.

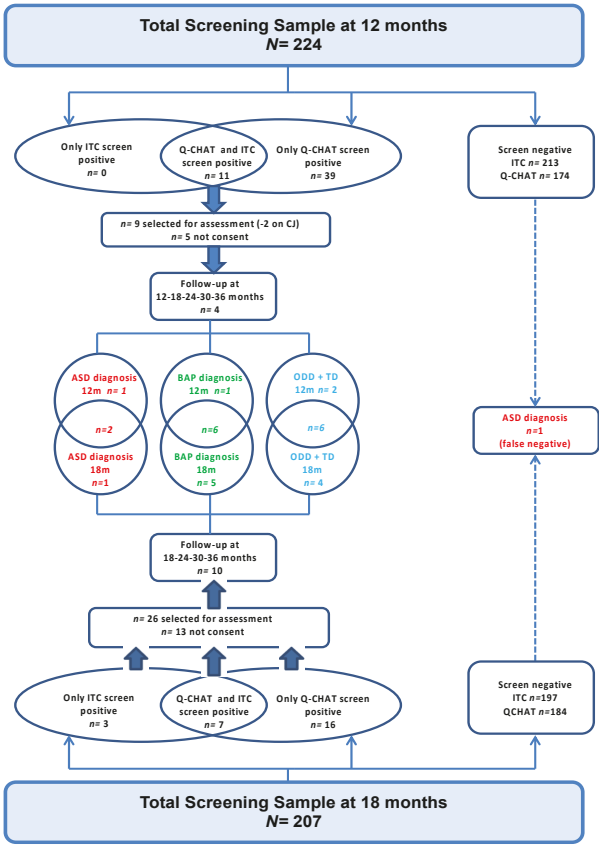


**Figure 1.** Flowchart of the project design. Project design. Two-stage screening approach at 12 and 18 months applied to the same sample. The intersections in the middle represent the children classified as autism spectrum disorder (ASD) ($n$ = 2), broader autism phenotype (BAP) ($n$ = 6), Other non-spectrum developmental disorders (ODD) + typical development (TD) ($n$ = 5) at the final outcome of 36 months. On the right the only false-negative case diagnosed as ASD at 36 months. I-TC: Infant–Toddler Checklist; Q-CHAT: Quantitative Checklist for Autism in Toddlers.

Neurodevelopmental disorders of known genetic etiology and significant vision, hearing, motor or physical problems have been identified as exclusion criteria. Two children were excluded from the study at the 12 months' data point because they were affected with a genetic disorder characterized by global developmental delays and dysmorphic features. For the diagnostic follow-up evaluations, 9 children at 12 months and 26 children at 18 months were recruited respectively. Among those who

respected the recruitment criteria, only 4 out of 9 children at 12 months, and 13 out of 26 children at 18 months were included in the study. Therefore, approximately half of the parents did not consent to the diagnostic assessment; additionally, 3 out of 13 children recruited at 18 months left the study after the 24 months follow-up visit because the parents did not recognize any risk for their child's development. At the last follow-up visit at 36 months, there were only 14 children who fully participated until the end of the study and received a final diagnosis. ASD diagnosis was confirmed based on the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (*DSM-5*) criteria [1] and the ADOS-2 [21], administered by experienced clinicians trained in research reliability.

### *2.2. Participants*

At 12 months, 224 toddlers were enrolled in the study. Of these, 207 toddlers repeated the screening at 18 months. The outcome at 36 months is known for all the children, even those with negative screenings, because in case of any developmental problems, they would be sent for diagnostic evaluation by their pediatrician at the only diagnostic center in the Trieste area, located at the Division of Child Neurology and Psychiatry of the Institute for Maternal and Child Health— IRCCS "Burlo Garofolo" in Trieste, Italy—a Regional public Institute for Health care and scientific research.

### *2.3. Measures*

As screening tools, we used the Infant–Toddler Checklist (I-TC) and the Quantitative Checklist for Autism in Toddlers (Q-CHAT) to identify children at risk for autism spectrum disorder in a low-risk population. We expected to identify children with autistic symptoms or traits consistent with ASD diagnosis or with a BAP condition, versus children with Other non-spectrum Developmental Disorders (ODD) and children with typical development (TD). Children classified as BAP displayed autistic traits below the ASD threshold. The I-TC is a part of the Communication and Symbolic Behavior Scales Developmental Profile (CSBS DP) and is a broadband screener for communication delays of children between 12 and 24 months of age.

The I-TC is a screening questionnaire that investigates children's social communication through 24 questions clustered in: emotion and eye gaze, communication, gestures, sounds, words, understanding, object use. It can be downloaded from www.brookespublishing.com/resource-center/screening-and-assessment/csbs/csbs-dp/csbs-dp-itc [22]. With a cut off of the 10th percentile relative to population norms, a positive screen indicates risk for communication delay, but it does not discriminate between ASD and other developmental disorders.

The Q-CHAT is a 25-item questionnaire for caregivers testing children's autistic behaviors and traits in toddlers aged 18 to 24 months. Each Q-CHAT item is scored on a 5-point scale to assess frequency, typicality and severity of autistic behavior, through a dimensional-quantitative approach.

We chose the cut-off the score as 38 for both 12 and 18 months because, in Allison et al. [20], 80% of children with ASD had a cut-point of at least 38% versus 8% of children with typical development. Both screening tools have been translated into Italian with the back-translation mode.

The diagnostic assessment included a clinical observation conducted by the child neuropsychiatrist as well as the administration of the following diagnostic tools:

The *Autism Diagnostic Observation Schedule, Second Edition* (ADOS-2) is a semi-structured schedule that investigates different areas of ASD, including social communication, play and repetitive behaviors. In addition to the clinical judgment, ADOS-2 distinguishes between ASD and other delays or typical development. This instrument was used as a part of the diagnostic evaluation.

The *Bayley Scales of Infant and Toddler Development, Third Edition* [23] evaluates cognitive, language and motor skills in children between 0 and 42 months. This instrument was used as a part of the diagnostic evaluation.

### 2.4. Statistical Analysis

We carried out descriptive analyses in order to present the characteristics of the population considered. We subsequently carried out bivariate logistic regressions, considering positivity to ASD or BAP at 36 months of age as outcome and single Q-CHAT items and I-TC clusters as potential predictors, collected at 12 and 18 months of age. We also considered the summary scores resulting from the Q-CHAT and I-TC, both at 12 and 18 months of age, as potential predictors. Finally, we conducted two separate multivariate logistic regressions with Q-CHAT items and I-TC clusters, respectively, that resulted in significant association with the outcome at bivariate logistic regression. We, then, adopted a stepdown procedure in order to obtain two potentially predictive models, one with Q-CHAT items and the other with I-TC clusters. For each of these final models, we also generated Receiver Operating Characteristic (ROC) curves, calculated the respective Areas Under the Curves (AUC) and selected sensitivity and specificity cut-offs. All statistical analyses were performed using Stata/IC14.2 (Stata/IC 14.2 (StataCorp LLC, College Station, TX, USA).

## 3. Results

At the final diagnostic assessment of 36 months, we have identified three children with ASD and six children with BAP, three children with ODD (i.e., language delay) and three others with TD. Two out of the three children diagnosed with ASD were identified through the screening. The third child who had scored 37 at Q-CHAT at 18 months was a false negative at screening and was identified by his pediatrician and referred later to the autism evaluation center for diagnostic evaluation. The sample consisted of 224 children (female = 50%, $n$ = 113; male = 50%, $n$ = 111).

As shown in Table 1, the majority of parents held a high school diploma or higher educational qualification (mothers: 85%, $n$ = 191; fathers: 85%, $n$ = 191), with 53% of the mothers ($n$ = 119) and 39% of the fathers ($n$ = 88) holding at least a bachelor's degree. Seventy percent of the mothers ($n$ = 157) and 96% of the fathers ($n$ = 215) were employed at the time of the study.

**Table 1.** Socio-demographic characteristics of the sample ($n$ = 224).

| Variables. | Modalities | Mean (SD) or Number (%) |
|---|---|---|
| Sex, $n$ (%) | Males | 111 (50) |
| | Females | 113 (50) |
| Prematurity $n$ (%) | | 15 (7) |
| Twins $n$ (%) twin birth | | 4 (2) |
| Kindergarten attendance, $n$ (%) | | 64 (29) |
| Maternal age at delivery, years, mean (SD) | | 32.7 |
| Paternal age at delivery years, mean (SD) | | 36.1 |
| Maternal educational level, $n$ (%) | Elementary school | 1 (0.4) |
| | Middle school | 32 (14) |
| | High school | 72 (32) |
| | University degree | 119 (53) |
| Paternal educational level, $n$ (%) | Elementary school | 1 (0.4) |
| | Middle school | 32 (14) |
| | High school | 103 (46) |
| | University degree | 88 (39) |
| Maternal occupational status, $n$ (%) | Employed | 157 (70) |
| | Housewife | 63 (28) |
| | Other/missing | 4 (2) |
| Paternal occupational status, $n$ (%) | Employed | 215 (96) |
| | Unemployed | 2 (1) |
| | Other/missing | 7 (3) |

SD: standard deviation.

We analyzed the properties of the two screeners in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). We have dichotomized the sample into two groups: the ones with TD and ODD (called non ASD) and the ones with ASD diagnosis or BAP conditions (called ASD), as shown in Table 2.

**Table 2.** Contingency tables of positivity to ASD and BAP at 36 months and positivity to I-TC and Q-CHAT at 12 and 18 months.

| **12 Months ($n$ = 224)** | | | | | |
|---|---|---|---|---|---|
| | **I-TC** | | **Q-CHAT** | | |
| | **Negative** | **Positive** | **Negative** | **Positive** | |
| Non ASD | 206 (96%) | 9 (4%) | 171 (80%) | 44 (20%) | 215 (100%) |
| ASD | 7 (78%) | 2 (22%) | 3 (33%) | 6 (67%) | 9 (100%) |
| **18 Months ($n$ = 207)** | | | | | |
| Non ASD | 194 (98%) | 4 (2%) | 182 (92%) | 16 (8%) | 198 (100%) |
| ASD | 3 (33%) | 6 (67%) | 2 (22%) | 7 (78%) | 9 (100%) |

At 12 months. I-TC: Sensitivity 22%; Specificity 96%; Positive predictive value 18%; Negative predictive value 97%. Q-CHAT: Sensitivity 67%; Specificity 80%; Positive predictive value 12%; Negative predictive value 98%. At 18 months. I-TC: Sensitivity 67%; Specificity 98%; Positive predictive value 60%; Negative predictive value 98%. Q-CHAT: Sensitivity 78%; Specificity 92%; Positive predictive value 30%; Negative predictive value 99%.

At 12 months, we found that the specificity was high for both screeners, better for I-TC (96%) than Q-CHAT (80%), while the sensitivity was low for both, better for Q-CHAT (67%) compared to I-TC (22%). The value of PPV was slightly higher in I-TC (18%) compared to Q-CHAT (12%), whilst the percentage of NPV remained high for both I-TC (97%) and Q-CHAT (98%).

At 18 months, we found that the specificity remained high in both the screeners, equally in Q-CHAT (92%) and I-TC (98%), while the sensitivity increased moderately in both with a greater extent in Q-CHAT (78%) than the I-TC (67%). The PPV increased in I-TC (60%) and Q-CHAT (30%) and NPV remained high (I-TC: 98%; Q-CHAT 99%).

At this point, we tried to identify both for Q-CHAT and I-TC items and clusters that are more often associated with ASD diagnosis or BAP conditions at the final 36 months' outcome. We found that at 12 months, through a bivariate logistic regression analysis, 5 items of the Q-CHAT were significantly associated with positivity to ASD or BAP (i.e., 5, 6, 10, 19 and 20; $p < 0.05$). These items were considered in a multivariate logistic regression analysis; through a stepdown procedure, by eliminating non-significant items with the higher p-value one at the time, we obtained a model with only 3 statistically significant items: item 6 ("*Does your child point to share interest with you (e.g., pointing at an interesting sight)*?"), item 19 ("*Does your child use simple gestures (e.g., wave goodbye)?*") and item 20 ("*Does your child make unusual finger movements near his/her eyes?*") (Table 3).

**Table 3.** Results of the multivariate logistic regression analysis stepdown procedure on the association between diagnosis of ASD or BAP at 36 months and Q-CHAT items significantly associated at the bivariate logistic regression at 12 months of age (Items 5, 6, 10, 19 and 20).

| **Q-CHAT Items** | **Regression Coefficients** | **Odds Ratios** | **95% CI** | ***p*-Value** |
|---|---|---|---|---|
| 6 | 0.6480598 | 1.91 | 1.11–3.30 | 0.020 |
| 19 | 0.6180474 | 1.86 | 1.09–3.15 | 0.022 |
| 20 | 0.6285134 | 1.87 | 1.05–3.35 | 0.034 |
| constant | −5.987096 | | | |

C.I: Confidence Interval.

This model had an area under the receiver operating characteristic (ROC) curve (AUC) of 90.7% and a cut-off could be chosen with 100% sensitivity and 72% specificity (Figure 2).

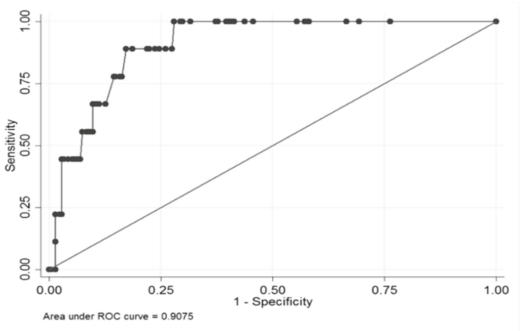


**Figure 2.** Area under the receiver operating characteristic (ROC) curve (AUC) of 90.7%.

At 18 months, the bivariate logistic regression analysis allowed us to identify 17 Q-CHAT items that were significantly associated to positivity to ASD or BAP (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 15, 16, 17, 19, 20, 23 and 25; $p < 0.05$). Again, starting from these items, we carried out a multivariate logistic regression adopting a stepdown procedure and obtained a model with 4 significantly associated items: item 10 ("*Does your child follow where you're looking?*"), item 14 ("*How easy is it for your child to adapt when his/her routine changes or when things are out of their usual?*"), item 19 ("*Does your child use simple gestures (e.g., wave goodbye)?*"), item 20 ("*Does your child make unusual finger movements near his/her eyes?*") (Table 4).

**Table 4.** Results of the multivariate logistic regression analysis stepdown procedure on the association between diagnosis of ASD or BAP at 36 months and Q-CHAT items significantly associated at the bivariate logistic regression at 18 months of age (Items 1, 2, 4, 5, 6, 7, 8, 9, 10, 14, 15, 16, 17, 19, 20, 23 and 25).

| Q_CHAT Items | Regression Coefficients | Odds Ratios | 95% CI | *p*-Value |
|---|---|---|---|---|
| 10 | 1.141131 | 3.13 | 1.03–9.52 | 0.044 |
| 14 | 3.942781 | 51.56 | 2.29–1161.08 | 0.013 |
| 19 | 2.510013 | 12.31 | 1.77–85.38 | 0.011 |
| 20 | 3.062373 | 21.38 | 2.40–190.41 | 0.006 |
| constant | −17.08924 | | | |

The final model had an AUC of 98.4%; we could keep an 100% sensitivity with a 93.9% specificity (Figure 3).

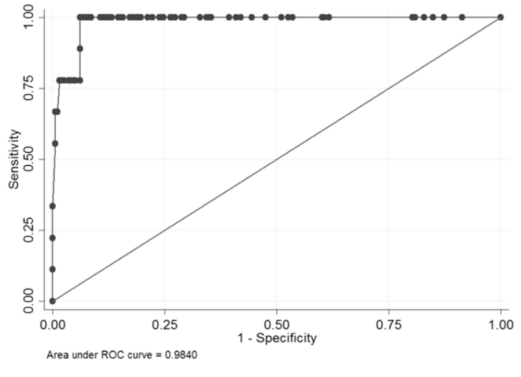


**Figure 3.** Area under the receiver operating characteristic (ROC) curve (AUC) of 98.4%.

Regarding the I-TC, the bivariate logistic regression analysis identified only one cluster at 12 months that was significantly associated with positivity to ASD or BAP: cluster 2 (Communication) (Odd ratio= 0.53; C.I. 95% = 0.009; *p*-value = 0.33–0.85). At 18 months, all seven I-TC clusters were significantly associated ($p < 0.05$). In the multivariate logistic regression model, after the application of the stepdown procedure, two clusters resulted in a significant association with the outcome: 1 (emotion and Eye Gaze) and 5 (Words) (Table 5).

**Table 5.** Results of the multivariate logistic regression analysis stepdown procedure on the association between diagnosis of ASD or BAP at 36 months and I-TC items significantly associated at the bivariate logistic regression at 18 months of age (Items 1 to 7).

| I-TC Items | Regression Coefficients | Odds Ratios | 95% CI | *p*-Value |
|---|---|---|---|---|
| 1 | −2.103662 | 0.12 | 0.03–0.43 | 0.001 |
| 5 | −1.62435 | 0.20 | 0.06–0.64 | 0.007 |
| constant | 13.96184 | | | |

This model had an AUC of 96.9% and maintaining a sensitivity of 100% could reach a specificity of 88% (Figure 4).

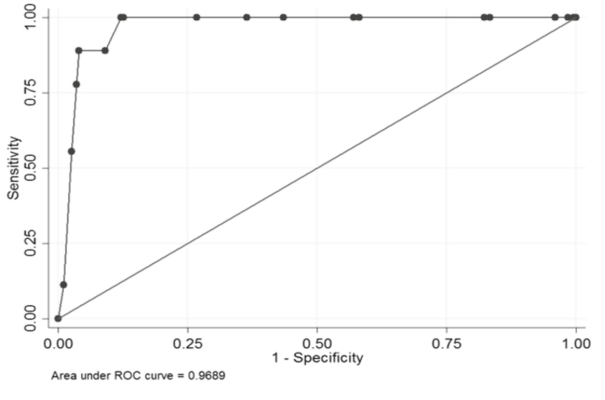


**Figure 4.** Area under the receiver operating characteristic (ROC) curve (AUC) of 96.9%.

Finally, in a multivariate logistic regression, we combined the statistically significant clusters and items at bivariate logistic regression from I-TC and Q-CHAT at 18 months (Q-CHAT items: 1, 2, 4 to 10, 14 to 17, 19, 20, 23 and 25; I-TC clusters: 1 to 7) and run a stepdown procedure. The model we obtained was based on three "predictors": I-TC clusters 1 and 5 and Q-CHAT item 20. This model had an AUC of 98.9% and obtained 100% sensitivity with and 95% specificity (Figure 5).

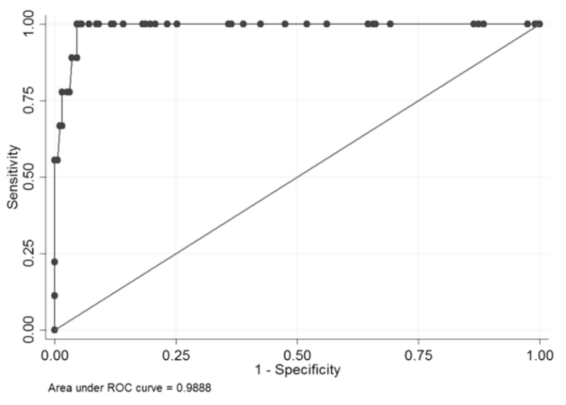


**Figure 5.** Area under the receiver operating characteristic (ROC) curve (AUC) of 98.9%.

We did the same analysis combining the statistically significant clusters and items at bivariate logistic regression from I-TC and Q-CHAT at 12 months, to run a stepdown procedure (Table 6).

**Table 6.** Results of the multivariate logistic regression analysis stepdown procedure on the association between diagnosis of ASD or BAP at 36 months and I-TC and Q-CHAT items significantly associated in the final models at 18 months of age (I-TC Items 1 and 5 and Q-CHAT Items 10, 14, 19 and 20).

| Items | Regression Coefficients | Odds Ratios | 95% CI | *p*-Value |
|---|---|---|---|---|
| I-TC item 1 | −2.969194 | 0.05 | 0.01–0.41 | 0.005 |
| I-TC item 5 | −2.012009 | 0.13 | 0.03–0.71 | 0.018 |
| Q-CHAT item 20 | 2.089171 | 8.08 | 1.86–35.1 | 0.005 |
| constant | 17.09517 | | | |

However, the significant clusters from I-TC were the first to be excluded, thus the results were solely based on Q-CHAT items as in the previously exposed model, shown in Table 4.

## 4. Discussion

We have described a screening protocol applied to a population of low-risk toddlers recruited at the clinics where mandatory vaccinations are carried out. Two different screeners were both administered at 12 and 18 months of age to identify the signs of risk for autism. Of all the children, we could know the outcome at 36 months because those who tested positive at 12 and at 18 months were longitudinally evaluated while any false negatives would have been referred by their pediatrician to the only available diagnostic center in the area. Therefore, we can affirm with reasonable certainty that, due to the screening carried out at two distinct stages, we were able to identify one case of ASD at 12 months and another one at 18 months. The third case of ASD was, unfortunately, the false negative who scored below 38 in the Q-CHAT and would likely be avoidable if we had adopted a risk "range" rather than using a pre-established cut-point. However, we made this choice based on data published by Allison et al. [20] in order to avoid recruitment of too many false positive cases.

We found that, at the age of 12 months, neither the Q-CHAT nor the I-TC has good overall sensitivity while, at 18 months, only the Q-CHAT has good sensitivity. Surely, more interesting was the result of the analysis that allowed us to identify some Q-CHAT items and some I-TC clusters, statistically more significant than the other items at 12 and 18 months, respectively. Of these two screening tools, we analyzed the properties and selected some items and clusters of items that are more sensitive to diagnostic identification. Such clusters may represent a brief measure to help determine whether a full diagnostic evaluation is needed.

In particular, the Q-CHAT has three items at 12 months and four items at 18 months with a very high sensitivity, which correspond to those questions that investigate the shared attention (items 1 and 6), the presence of simple communicative gestures (item 19) and the presence of stereotypical movements with fingers close to the eyes (item 20). Specifically, these last two items, items 19 and 20, remain significant at both 12 and 18 months as they maintain very high sensitivity and specificity at both ages. This finding would support previous research describing the presence of repetitive behaviors among children who go on to develop ASD as early as 12 months of age [24].

Regarding the I-TC instead, at 12 months, the only cluster significantly related to the outcome is communication (cluster 2) while, at 18 months, the clusters investigating areas, such as social engagement and shared attention (cluster 1) and verbal communication (cluster 5) appear. Moreover, at 18 months, these last two clusters of the I-TC, combined with item 20 of the Q-CHAT, constitute a model of three predictors with very high sensitivity and specificity. This confirms that the I-TC is a broadband screener which covers multiple developmental areas while the Q-CHAT seems more specific for autism and better discriminates among autism children, typical development and also from other developmental conditions, as suggested by Ruta et al. [25].

Also in our study it appears evident that it is more difficult to identify at 12 months any screening tools—or single items—that maintain a stable predictive value. This is the reason why we established as a recruitment criterion at 12 months that toddlers were positive for both screeners and that they were visited by a neuropsychiatrist expert in autism, in order to obtain a Clinical Best Estimate (CBE); while at 18 months being positive for only one of the two screeners, confirmed by clinical judgment, was enough. In this way, we were able to identify already at 12 months one out of three of the children who were diagnosed with ASD at the following diagnostic assessments and thus sent him for early intervention. The recruitment strategy we adopted in our study could be recommended in order to limit the rate of false cases, which is certainly higher at 12 months than at 18 months. Furthermore, our results suggest that it would be possible to administer at 12 months only the three most sensitive Q-CHAT items and at 18 months the short version of three predictors to identify a risk for ASD, being aware that in very young children (12–14 months) it is correct to assume a risk; it is not yet possible to make a diagnosis. However, these results seem to be promising and worthy of future confirmation in larger studies.

In our model, we believe that the screening combined with a mandatory procedure, due to vaccination policies in Italy, can optimize the spread of screening to a wider low-risk population. In addition, as an unexpected consequence, a large majority of parents declared that they had been given an educational opportunity and felt that they had gained a greater awareness in monitoring their child's development. Perhaps, this active participation by parents could have been positively influenced by the high level of parents' education, especially of mothers (as can be seen from the socio-demographic data table). Moreover, the repetition of screening helps to identify a wider population at developmental risk composed of children with late onset of symptoms and false positive cases with other neurodevelopmental disorders. Screening conducted too early may not be able to distinguish ASD from other developmental delays or even typical developmental delays as it may not detect cases of plateau or regression, which are about 30% of individuals with ASD [26,27]. Only a longitudinal diagnostic assessment can confirm the ASD diagnosis and provide major details about the different developmental trajectories [28,29]. Additionally, in case of false positives, which often result in other non-spectrum disorders, early recognition can mean a better prognosis and earlier access to treatment. Among these cases, we also include BAP, which is not a diagnostic entity due to much milder difficulties than ASD. However, BAP in early childhood has been described as social and communication difficulties and rigidity of behaviors; little or nothing is known about its long-term evolution. We can hypothesize that subtle ASD signs at early ages could become more evident at school age under an increasing social demand [30]. Therefore, it is crucial to know more about the long-term consequences of certain early developmental patterns and to provide guidance to parents.

Our study presents some limitations including a small sample size and a limited geographical area. Therefore, our study should be replicated with a larger sample size in a larger geographical area. Despite the limitations, if our results are confirmed with a bigger sample, our model could be used as a screening procedure in the Italian public health system.

In conclusion, the results of our study show that atypical aspects of development can be identified as early as the first year of life and that two different screening tools, such as the I-TC and the Q-CHAT, combined together and administered in a two-stage approach can help to identify children at risk for ASD symptoms or autistic traits, perhaps even using reduced versions consisting of a few questions extrapolated from both screeners.

**Author Contributions:** R.D. participated in the study design and coordination, provided clinical oversight for data collection and interpretation, draft the manuscript; C.C. conceptualized the study and supervised the manuscript; L.M. performed statistical analysis of the data; M.B. participated in data collection and processing, and contributed to the literature review; A.M. was involved in processing and interpretation of the data; G.B. contributed to the literature review and helped draft the manuscript; M.C. supervised the coordination of the study. All authors have read and approved the final manuscript.

**Funding:** This study was funded by the Italian Ministry of Health (Ricerca Corrente of Institute for Maternal and Child Health-IRCCS- "Burlo Garofolo"-Trieste-Italy).

**Acknowledgments:** We wish to thank the families participating in our study and Daniela Vidoni and Cesarino Zago—S.C. Tutela Salute Bambini Adolescenti Donne e famiglie, Trieste—for allowing us to collaborate with public health vaccination centers where parents were given the screening questionnaires. We are grateful to Megan Puckett and Annes Kim for the English editing of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

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# Early Screening of the Autism Spectrum Disorders: Validity Properties and Cross-Cultural Generalizability of the First Year Inventory in Italy

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Received: 13 January 2020; Accepted: 13 February 2020; Published: 18 February 2020

**Abstract:** This study examined the cross-cultural generalisability of the First Year Inventory (FYI) on an Italian sample, testing its construct validity, consistency, and structural validity. Six hundred ninety-eight parents of children aged 11–13 months completed the questionnaire. Similarities between analyses of Italian and American/Israeli samples were found, as were demonstrations of the instrument's construct validity and internal consistency with both groups. The original factorial structure was not demonstrated; thus, a new factorial structure was tested, and a short version of the FYI was demonstrated via confirmatory factor analysis. The findings supported the generalisability of the Italian version of the FYI and its validity. The FYI may aid in medical decision-making on further steps for referral of the child to an early diagnostic assessment.

**Keywords:** First Year Inventory; autism spectrum disorders; early screening; risk; cross-cultural generalisability; validity

## 1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterised by (a) persistent deficits in social communication and interaction and (b) restricted and repetitive patterns of behaviours, interests, and/or activities [1]. Recent epidemiological data [2] suggested that the prevalence of ASD reaches the proportion of 1/59 at age 4. To promote early detection of the risk of ASD, as recommended also by the American Academy of Pediatrics [3], several researchers [4–8] developed ad hoc measures for children under 24 months of age that are able to identify behaviours deviating from typical development.

In this vein, a recent systematic review [9] identified 16 Level 1 and 2 screening measures for the early detection of signs of ASD: 4 observational checklists, 2 interviews, and 10 questionnaires. Level 1 screening tools have been developed for the general population to detect children at risk of developmental disorders, including ASD. Level 2 screening measures have been developed to

detect children who are at risk for ASD, since they are already referred to the health service for developmental concerns (i.e., low-risk children) or because they are siblings of children with ASD (i.e., high-risk children). This review identified five promising instruments: the First Year Inventory (FYI) [8], the Modified-CHecklist for Autism in Toddler and its revised/follow-up form (M-CHAT and M-CHAT-R/F) [6,10], the Parental Observation of Early Markers Scale (POEMS) [11], and the Quantitative-CHecklist for Autism in Toddler (Q-CHAT) [4]. Analyses of the psychometric properties of these measures evaluated them as good. At the same time, however, the authors stressed that, for several such measures, further validation studies were needed to evaluate certain methodological properties that, as yet, were not adequately investigated.

The highest number of validation studies retrieved in the literature were for the M-CHAT and the M-CHAT-R/F [6,10,12–26]. The Q-CHAT has been validated by five studies [4,27–30] and the FYI by five studies [8,31–34]. The POEMS has been validated by one study [11].

The M-CHAT and the M-CHAT-R/F can be administered from 16 months of life, the POEMS from 1–24 months of life, the Q-CHAT is administrable when the child is 18 months old, and the FYI when he/she is 11–13 months old. The POEMS requires more administration time as it uses multiple parental observations. The present study focused on the FYI since it allows the earliest screening but—in contrast to the POEMS—requires less administration time and can be completed by parents during regular well-child visits as part of pediatric surveillance.

*The First Year Inventory: Measure Description and Critical Analysis of the Validation Studies*

The FYI is a Level 1 screening measure designed to detect the ASD risk on the general population. It was developed through a systematic review of the literature conducted by Reznick and colleagues [8], who identified a list of behaviours comprised in the two core diagnostic criteria of the ASD (i.e., socio-communication and social interaction deficit and restricted, repetitive patterns of behaviour) [1]. Specifically, the authors analysed several retrospective studies and descriptive reports provided by parents, which assessed the first months of life of children with a later diagnosis of ASD, and prospective studies on children who had an older sibling with a diagnosis of ASD. As the authors highlighted, two sets of behaviours, clustered in two categories labelled 'Social–Communication' and 'Sensory–Regulatory Functions', detect children who are at risk of developing, at an early age, an ASD [8]. The Social–Communication domain was further differentiated into four constructs (Social Orienting & Receptive Communication, Social-Affective Engagement, Imitation, and Expressive Communication) as well as the Sensory–Regulatory Functions domain (Sensory Processing, Regulatory Patterns, Reactivity, and Repetitive Behaviors). For a detailed description of the domains and constructs, please refer to the Appendix of Reznick and colleagues' paper [8].

The 63 items of the FYI include 46 questions with response options on a four-point Likert scale (from 1—'never'—to 4—'often') and 14 items with answers in a three or four ad hoc multiple-choice format (see Appendix in [8]). Three additional open-ended questions were on (a) the number of consonants used by the child (Item 61); (b) parental concerns or interests about the child's development (Item 62); and (c) the presence of a specific medical condition (Item 63). Item 61 is scored from 0 (i.e., if the child uses more than three consonants) to 2 points (if the child uses only one or any consonants). The two last open-ended questions (Items 62 and 63) did not receive a score because they were used for qualitative evaluation.

This first study of the FYI was on an American sample (N = 1300) selected from the general population [8], with the purposes of (a) defining the scoring procedure; (b) identifying the risk cut-offs; and (c) evaluating the factorial structure of the instrument. With regard to the scoring procedure, according to the response distribution of the sample, the authors assigned 0 or 1 point to the answers corresponding to behaviours with the highest frequency expected in typically developing children (i.e., low risk). For example, Item 1 ('Does he/she look at you when his/her name is called?') received 0 or 1 point when the answer is respectively 'always' or 'sometimes' because it is expected that a typically developing child looks at the person who calls his/her name. Two points are assigned to answers

that have either low frequency (<5%) or correspond to behaviours unusual in typically developing children. For Item 1, the answers 'never' and 'seldom' receive 2 points because they represent unusual behaviours for a typically developing child.

To identify the cutoffs of risk, the authors [8] observed that the distribution had a chi-squared shape and identified a significant shape inflection corresponding to the score of 17 (at the 95th percentile of the distribution). Finally, they conducted an exploratory factor analysis (EFA), applying the principal factor method followed by a promax (oblique) rotation. The EFA accounted for six factors corresponding to four constructs of the Socio-Communication domain (Social-Affective Engagement—six items, Imitation—four items, Social Orientation—two items, and Expressive Communication—two items) and two constructs of the Sensory–Regulatory Functions domain (Regulatory Patterns—four items and Repetitive Behaviors—eight items). Thirty items did not load for any factor or loaded for more than one factor. To accomplish the broader goal of developing a measure for the early detection of ASD, the authors sorted the 61 items into the hypothesised eight constructs and two domains according to the theoretical model. After the EFA, each of the nonassigned items was allocated to a construct if the item theoretically fitted with that construct, the item–total correlation was higher than 0.30, and the change to Cronbach's alpha was negligible. After that procedure, nine items were assigned to an uncategorised group because they did not fit any of these criteria.

The FYI was tested in four other studies [31–34]. One [33] was a follow-up investigation of the Reznick and colleagues' sample [8] developed three years later. Two were retrospective studies on an American [34] and an Italian sample [32] of children with ASD. Finally, a more recent study [31] was published using an Israeli sample from the general population.

In the following section, we reported a critical comparison between validation studies.

All validation studies carried out analysis on children's (gender and family size) and parents' (educational level, ethnicity, and marital status) socio-demographic variables. With regard to the children's gender, all validation studies found a similar result: all males reached a higher score than females, both in the general [8,32] and clinical [35] population. Only Reznick and colleagues [8] found no significant impact of the family size variable on FYI score. With regard to the parental variables, only two studies [8,32] evaluated them. Specifically, both Reznick and colleagues [8] and Ben-Sasson and [32] found a negative and significant impact of low maternal educational level on FYI score. For this reason, both validation studies suggested rewriting several items. Furthermore, the study by Reznick and colleagues [8] found a significant and positive impact on FYI score for black mothers, whereas Ben-Sasson and Carter [32] found a significant and positive impact of single status mothers on screening measure score. As suggested by these authors [8,32], these variables could be monitored by researchers and professionals to interpret the FYI score adequately.

With regard to the questionnaire psychometric properties, it was worth noting possible detected similarities and differences between the validation studies. The convergent validity was demonstrated by two studies [32,34]. The first study [34] carried the analysis on a sample of the general population recruited by Reznick' study [8]; the second study [32] analyzed a sample of the Israeli general population. Both validation studies administered the observation and standardized measures to assess the child' autistic traits (ADOS 2—Autism Diagnostic Observation Schedule-Second Edition—and AOSI—Autism Observation Scale for Infants [36]—respectively) and his/her global functioning (MSEL). Furthermore, both validation studies suggested developing a short version of the FYI. Only Turner-Brown and colleagues [34] examined the accuracy of the screening measure applying a Receiver Operating Characteristic (ROC) analysis: they stated that the combined score on Social–Communicative and Sensory–Regulatory Functions domains was the optimal threshold to detect child at risk at 12 months. In addition, Muratori and colleagues [33] evaluated the FYI accuracy on a clinical sample, and they stated that a two-domains approach of social-communicative and total domains was the optimal threshold to detect cases of early-onset autism. Finally, only Reznick and colleagues [8] demonstrated the questionnaire structural validity and carried out an Explorative Factor Analysis (EFA). As anticipated above, the factorial structure was developed according to the results of two different statistical analysis:

the EFA and the Item–Total Correlation (ITC). Nevertheless, this statistical strategy was not adequate to define a factorial structure, and not one validation study carried out a Confirmatory Factor Analysis (CFA).

According to the systematic review findings and the present critical analysis of the validation studies of the FYI, the measure seems to show some promising characteristics and several limitations. The FYI is an effective tool requiring little administration time that can be applied starting from 11 months of life, both in general populations and those at risk. Therefore, the FYI is a cost-effective measure, appropriate for administration to parents during regular well-child visits as part of pediatric surveillance. Finally, according to the longitudinal research [33], the instrument seems to be an efficient measure for detecting behaviours that deviate from those characterising typical development (and, as such, can be a sign of the risk of ASD).

Nevertheless, the above-mentioned studies have several limitations. First, the cross-cultural generalisability of the FYI was studied on Israeli children. One study [32], involving Italian children, used a retrospective design. It is well known that parental memories may influence the quality of data derived through retrospective methods [37]; thus, further studies are needed to study the cross-cultural generalisability of this measure in a non-American sample. Second, the factorial analysis of the FYI [8] has not confirmed a structure based on the expected eight constructs. It should be noted that the authors did not report the results from the EFA (i.e., factor loadings, percentage of variance explained), and the final structure of the questionnaire was derived from a combination of evidence from the item–total correlations and what they theoretically expected to find. Establishing a psychometrically sound factorial structure of the FYI is not a secondary issue since the calculation of the risk cutoff is based on it. Finally, none of the other studies [31–34] analysed the factorial structure of the FYI, but rather took for granted what Reznick and colleagues [8] had found. Thus, further demonstrations of the factorial structure are particularly needed.

Therefore, the general aim of the current study was to conduct a screening of the signs of risk of ASD, applying the FYI on an Italian sample (from the general population) undergoing regular well-child visits as part of pediatric surveillance. The study purposes were to (a) examine the cross-cultural generalisability of the FYI, comparing the Italian findings with those of US and Israeli samples (specifically, comparisons of the analyses of socio-demographic variables, response distributions, and cut-offs); (b) demonstrate the construct validity of the FYI; and (c) demonstrate the internal consistency and structural validity of the FYI.

## 2. Materials and Methods

### *2.1. Procedure*

The study was carried out in a large urban area in the south of Italy. The Ethical Committee of the Local Public Health Service gave its approval for this research (n° 528/8 March 2017). One hundred fifteen paediatricians of the local public health service received via mail a description of the research project, with a request to collaborate with it. Sixty-four of them (55.6%) participated in the research and received instructions for the recruitment of participants. All families treated by those paediatricians with a child born between February and September of 2016 were invited to participate in the study ($n$ = 800). They received a description of the research project and signed informed consent. Data collection was conducted when the parents were at the paediatrician's office (in a quiet place before the visit); the paediatrician was not present during the administration of the questionnaire.

### *2.2. Measure*

Socio-Demographic Variables. The first part of the FYI allows identification of the following information: the child's gender, date of birth, weight at birth, order of birth, term birth vs. preterm birth, parents' marital status, their educational level, and their ethnicity. Finally, information was collected as well on who completed the questionnaire (e.g., mother, father, or both). Early identification

of signs of risk of ASD. The 63 items of the FYI [8] (Italian translation by Muratori and Narzisi, 2009) allow evaluation of the child's functioning within two domains: Social–Communication and Sensory–Regulatory Functions. Each domain consists of four constructs. The Social–Communication domain includes the constructs of Social Orienting & Receptive Communication (nine items), Social–Affective Engagement (eight items), Imitation (six items), and Expressive Communication (five items). The Sensory–Regulatory Functions domain includes the constructs of Sensory Processing (six items), Regulatory Patterns (four items), Reactivity (three items), and Repetitive Behaviors (eleven items). According to Reznick and colleagues [8], the final score was calculated through a weighted average of the raw score for each construct and domain. a total score was calculated as an average of the two domains, with higher scores indicating higher risk.

### *2.3. Participants*

The convenience sample was composed of 698 returned questionnaires with a response rate of 86.1%. Forty-one questionnaires were excluded from the analyses because they were completed by mothers of children with Down's Syndrome ($n$ = 2) or by mothers of preterm children (i.e., born before the 37th gestation week; $n$ = 39). Those children were excluded from the sample since the study purpose was to validate the FYI as a Level 1 screening measure administrable to the general population, that is, children not referred for other developmental concerns. Specifically, the two children with Down's Syndrome were excluded from the sample because of their genetic disease. Furthermore, the 39 preterm children were excluded since—as in [8] and [32]—they were too immature at 12 months to be evaluated on social and behavioural functioning.

The final sample was comprised of 657 questionnaires (Figure 1) completed by mothers (69.9%), fathers (5.3%), or both parents together (24.2%) when the children were from 11 to 13 months old (M = 12.4 months; SD = 1 month). Three hundred forty-one of them were boys, 309 were girls. The toddlers' mean weight at birth was 3.32 kg (SD = 0.51; range 3–4.93 kg); 40.3% of the children were first-born, and 43.7% were second-born or more. The mothers' mean age was 33.83 (SD = 5.6; range 18–49), and their educational level was low (up to eight years of education) for 26.9% and high (nine or more years of education) for 73.8%. The fathers' mean age was 37.42 (SD = 6.4; range 19–67), and their mean of the educational level was low (up to eight years of education) for 32.1% and high (nine or more years of education) for 61.3%. The majority of the parents were married (92.8%), whereas 6.4% were single or divorced. The parents were European–White (88.1% of the mothers; 85.7% of the fathers), African (0.6% of the mothers; 1.1% of the fathers), or Asian (1.1% for mothers; 0.3% of the fathers).

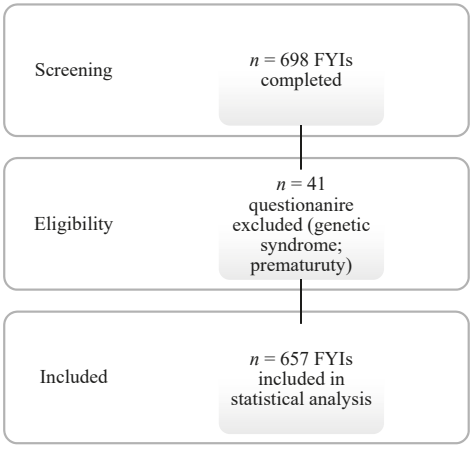


**Figure 1.** Flowchart of study sample and design.

### *2.4. Analytic Strategy*

Independent sample t-tests were carried out to analyse the differences in the two domains of the FYI (Social–Communication and Sensory–Regulatory Functions), the total score, and the eight constructs based on the socio-demographic variables. When a difference was found as statistically significant, a Cohen's *d* was reported. To compare the Italian and American (or Israeli) response distributions, a chi-square analysis was run for each item. The null hypothesis ($H_0$), that the response distribution of the Italian and American sample (or Israeli) for each item was not different, is what we aimed to demonstrate. Thus, a nonsignificant chi-square is a demonstration that the distributions are comparable. The analyses were conducted in SPSS v.25.

The data were screened to investigate the missing data distribution, normality distribution, and outliers. Exploratory factor analyses (EFA) and confirmatory factor analyses (CFA) through SEM (Structural Equation Modelling) were carried out in Mplus v.8 applying WLSMV because the data were ordinal. Geomin rotation was applied to the EFAs with the Weighted Least Square Mean and Variance (WLSMV) as estimator since the data were ordinal and missing data were also found. The Kaiser–Meyer–Olkin (KMO) statistic was computed on the 60 items of the FYI to evaluate if the data were suitable the data for the factor analysis.

## 3. Results

### *3.1. Preliminary Analysis*

Less than 5% of the socio-demographic variables and less than 1.7% for the items of the FYI were missing. Among the latter, those with the highest percentages of missing data were Item 40 (1.7%), Item 5 (1.3%; 'Does your baby seem to have trouble hearing?'), and Item 16 (1.1%; 'Is it easy to understand your baby's facial expressions?'). The 'Little's missing completely at random' test was significant, $\chi^2$ (3367) = 4008.438; $p$ = 0.000; this means that missing data were nonrandomly distributed. For this reason, and given the low percentages, they were not imputed. Comparing our missing patterns with those of the US sample [8], only Item 40 ('Do your baby's eyes line up together when looking at an object?') had a similar percentage of missing data (1.7% in the Italian sample and 2% in the American sample). For all the other items, we had less missing data than the US sample.

### *3.2. Generalisability*

Analyses on the socio-demographic variables. The t-tests showed no significant effects by the childbirth order (i.e., first-born = 40.3%; second-born or more = 43.7%) on the FYI domains, the total score, or the eight constructs. With regard to the children's gender, the t-test showed a significant difference on the Reactivity construct. Boys obtained higher scores than girls. Boys reached higher scores also on the two domains and on the total score. Table 1 shows the results of the t-tests, with means and standard deviations.

**Table 1.** Independent-sample t-test by gender on the FYI domains, total score, and constructs.

| | Males M(ds) | Females M(ds) | *t* | Cohen's *d* |
|---|---|---|---|---|
| **Social–Communication domain** | 2.83 (3.08) | 2.64 (3.03) | $t(648) = 0.766$ | - |
| Social orienting & receptive communication | 1.13 (2.69) | 1.26 (2.84) | $t(648) = -0.582$ | - |
| Social-affective engagement | 2.02 (3.70) | 2.23 (4.01) | $t(648) = -0.691$ | - |
| Imitation | 1.44 (3.79) | 1.29 (4.25) | $t(648) = 0.467$ | - |
| Expressive communication | 6.71 (7.79) | 5.79 (7.17) | $t(648) = 1.567$ | - |
| **Sensory–Regulatory Functions domain** | 4.07 (4.01) | 3.49 (3.65) | $t(648) = 1.921$ | - |
| Sensory processing | 3.71 (5.14) | 3.72 (5.32) | $t(648) = -0.029$ | - |
| Regulatory patterns | 4.54 (9.35) | 3.72 (7.8) | $t(643.626) = 1.224$ | - |
| Reactivity | 2.08 (5.32) | 1.26 (4.42) | $t(643.169) = 2.145$ * | 0.17 |
| Repetitive behaviors | 5.94 (7.02) | 5.25 (6.41) | $t(648) = 1.303$ | - |
| **Total score** | 3.44 (2.68) | 3.06 (2.52) | $t(648) = 1.867$ | - |

Note: * $p < 0.05$.

Considering the parental socio-demographic variables, the t-tests showed differences for maternal educational level and marital status. Specifically, mothers with a low educational level (up to eight years of education), compared to those with high educational level (nine or more years of education), obtained higher scores in the two FYI domains, the total score, and all constructs, with the exception of Social–Orienting and Receptive Communication (part of the Socio-Communication domain) and Regulatory Patterns (part of the Sensory–Regulatory Functions domain) constructs. Table 2 shows the results of these analyses.

**Table 2.** Independent-sample t-tests by maternal educational level on the FYI domains, total score, and constructs.

| | Low Educational Level M(ds) | High Educational Level M(ds) | *t* | Cohen's *d* |
|---|---|---|---|---|
| **Social–Communication domain** | 3.45 (3.76) | 2.54 (2.80) | $t(197.576) = 2.736$ * | 0.27 |
| Social orienting & receptive communication | 1.04 (2.54) | 1.22 (2.82) | $t(630) = -0.695$ * | 0.07 |
| Social–affective engagement | 2.98 (4.80) | 1.82 (3.46) | $t(194.269) = 2.719$ * | 0.27 |
| Imitation | 1.90 (5.23) | 1.26 (3.60) | $t(189.821) = 1.378$ | - |
| Expressive communication | 7.90 (8.37) | 5.84 (7.19) | $t(215.355) = 2.692$ * | 0.26 |
| **Sensory–Regulatory Functions domain** | 4.65 (4.15) | 3.59 (3.81) | $t(630) = 2.895$ * | 0.06 |
| Sensory processing | 4.90 (6.18) | 3.45 (4.93) | $t(205.617) = 2.594$ * | 0.26 |
| Regulatory patterns | 4.03 (7.93) | 4.37 (9.20) | $t(630) = -0.406$ | - |
| Reactivity | 2.52 (5.59) | 1.44 (4.59) | $t(208.993) = 2.121$ * | 0.21 |
| Repetitive behaviors | 7.16 (7.51) | 5.10 (6.33) | $t(212.843) = 3.028$ * | 0.30 |
| **Total score** | 4.05 (3.03) | 3.06 (2.45) | $t(207.011) = 3.615$ * | 0.36 |

Note: * $p < 0.05$.

Mothers without a partner showed higher scores (M = 8.03; ds = 7.90) on the Repetitive Behaviors construct (part of the Sensory–Regulatory Functions domain) than mothers with a partner (M = 5.40; ds = 6.48), $t(44.876) = -2.109$, $p = 0.041$.

Comparisons between distributions. We aimed to demonstrate the null hypothesis ($H_0$), that the percentage of response distribution for each item for the Italian and American (see Table 3) and Israeli (see Table 4) samples was not different. Indeed, the first column of the Table 3 reports the content of the items, and the second to the fifth columns report the percentages for each response for the two samples.

**Table 3.** Chi-square comparison between American (AS; Reznick et al., 2007) and Italian (ItS) sample distribution (%).

| | Never | | Seldom | | Sometimes | | Often | |
|---|---|---|---|---|---|---|---|---|
| | **ItS** | **AS** | **ItS** | **AS** | **ItS** | **AS** | **ItS** | **AS** |
| 1. Does your baby turn to look at you when you call your baby's name? | 0 | <1 | 0.3 | 1 | 2.3 | 8 | 97.4 | 91 |
| 2. Does your baby seem bothered by loud sounds? | 21.2 | 8 | 25.3 | 39 | 41.6 | 46 | 11.7 | 7 |
| 3. Does your baby seem overly sensitive to your touch (for example, fuss or pull away when you touch him or her)? | 71.2 | 64 | 19.4 | 31 | 6.2 | 5 | 2.4 | <1 |
| 4. During familiar games like "I'm gonna get you," does your baby get excited because he or she knows what will happen next? | 1.4 | <1 | 0.6 | <1 | 7 | 8 | 90.7 | 92 |
| 5. Does your baby seem to have trouble hearing? | 97 | 94 | 1.2 | 5 | 0.2 | 1 | 0.5 | <1 |
| 6. When you and your baby are facing each other, does your baby turn his or her eyes to avoid looking at you? | 81.7 | 53 | 11.9 | 30 | 4.7 | 15 | 1.1 | 2 |
| 7. In new or strange situations, does your baby look at your face for comfort? | 3.3 | 1 | 7.2 | 6 | 28.6 | 40 | 60.4 | 53 |
| 8. Does your baby ignore loud or startling sounds? | 65.3 | 34 | 20.5 | 42 | 10.7 | 21 | 2 | 3 |
| 9. Does your baby spit out certain textures of foods, such as lumpy or chunky pieces? | 25.3 | 11 | 18.4 | 25 | 35.3 | 48 | 19.6 | 16 |
| 10. When you point to something interesting, does your baby turn to look at it? | 0.5 | 1 | 1.4 | 4 | 13.2 | 39 | 83.9 | 56 |
| 11. Is your baby content to play alone for an hour or more at a time? | 35.6 | 27 | 28.2 | 29 | 22.2 | 31 | 13.1 | 13 |
| 12. Does your baby look at people when they begin talking, even when they are not talking directly to your baby? | 0.3 | <1 | 1.4 | 3 | 15.2 | 44 | 83 | 53 |
| 13. Does your baby rock his or her body back and forth over and over? | 61 | 54 | 14.8 | 24 | 16.7 | 15 | 7 | 7 |
| 14. Does your baby look up from playing with a favorite toy if you show him or her a different toy? | 1.7 | <1 | 3.8 | 2 | 31.1 | 39 | 62.7 | 59 |
| 15. Does your baby get upset when you need to switch your baby from one activity to another one? | 34.1 | 7 | 28.5 | 35 | 30 | 53 | 7 | 5 |
| 16. Is it easy to understand your baby's facial expressions? | 0.8 | <1 | 0.9 | 1 | 6.1 | 14 | 90.7 | 85 |
| 17. Does your baby forcefully press his or her face, head, or body against people or furniture? | 79.6 | 38 | 10 | 27 | 7.6 | 24 | 2.1 | 11 |
| 18. Does your baby smile while looking at you? | 0.2 | <1 | 0.2 | <1 | 5.2 | 9 | 94.2 | 91 |
| 19. Does your baby try to get your attention to show you something interesting? | 1.4 | 7 | 3.5 | 16 | 26.6 | 40 | 67.9 | 37 |
| 20. Does your baby try to get your attention to play games like peek-a-boo? | 3.8 | 5 | 5 | 15 | 25 | 41 | 65.4 | 39 |
| 21. Does your baby try to get your attention to obtain a favorite toy or food? | 1.4 | 2 | 2.3 | 9 | 14.6 | 32 | 81.1 | 57 |
| 22. Does your baby try to get your attention to play physical games, like swinging, tickling, or being tossed in the air? | 4.6 | 10 | 9.1 | 23 | 33.9 | 40 | 51.9 | 26 |
| 23. When your baby is awake and you pick him or her up, does your baby's body feel loose or floppy? | 87.4 | 81 | 7 | 14 | 3.3 | 4 | 1.4 | 1 |
| 24. Does your baby copy or imitate you when you make sounds or noises with your mouth? | 0.9 | 1 | 2.6 | 4 | 22.7 | 32 | 73.5 | 63 |
| 25. Does your baby copy or imitate your actions, like sticking out your tongue, clapping your hands, or shaking your head? | 0.9 | <1 | 1.5 | 2 | 9.9 | 23 | 87.5 | 75 |
| 26. Does your baby copy or imitate you when you do something with a toy or object, like shaking a rattle or banging a spoon on the table? | 0.8 | <1 | 1.1 | 1 | 9.7 | 22 | 88.3 | 77 |

**Table 3.** *Cont.*

| | Never | | Seldom | | Sometimes | | Often | |
|---|---|---|---|---|---|---|---|---|
| | **ItS** | **AS** | **ItS** | **AS** | **ItS** | **AS** | **ItS** | **AS** |
| 27. Is it difficult to calm your baby once he or she becomes upset? | 33.9 | 20 | 41.7 | 62 | 19.9 | 17 | 4.1 | 1 |
| 28. Are your baby's sleeping and waking patterns regular from day to day? | 1.7 | 1 | 6.1 | 4 | 11.8 | 20 | 80.2 | 75 |
| 29. Does your baby try to get your attention by making sounds and looking at you at the same time? | 3.7 | 1 | 6.4 | 4 | 26.5 | 30 | 62.7 | 65 |
| 30. Does your baby get stuck doing a simple activity over and over? | 79.3 | 36 | 14.6 | 45 | 4 | 16 | 1.1 | 3 |
| 31. Does your baby seem interested in other babies his or her age? | 0.5 | <1 | 0.8 | 5 | 9.5 | 28 | 89 | 67 |
| 32. Does your baby babble by putting sounds together, such as 'ba-ba', 'ga-ga-ga', or 'ba-dee'? | 8.1 | <1 | 3.2 | 1 | 11.7 | 8 | 76.6 | 91 |
| 33. Does your baby enjoy staring at a bright light for long periods of time? | 62.56 | 49 | 22.1 | 32 | 11.7 | 15 | 2.9 | 4 |
| 34. Does your baby use gestures such as raising arms to be picked up, shaking head, or waving bye-bye? | 0.2 | <1 | 0.3 | 3 | 3.7 | 12 | 95.9 | 85 |
| 35. When you say "Where's (a familiar person or object)?" without pointing or showing, will your baby look at the person or object named? | 0.6 | 4 | 2 | 10 | 13.7 | 35 | 83.3 | 51 |
| 36. Does your baby use the first finger and tip of the thumb to pick up a very small object like a raisin or a Cheerio? | 1.4 | <1 | 1.9 | 1 | 5.9 | 5 | 90.4 | 94 |
| 37. Does your baby seem to get stuck on playing with a part of a toy (such as an eyeball, label, wheel or tag), instead of the whole toy? | 16.1 | 14 | 18.1 | 32 | 34.1 | 39 | 31.4 | 15 |
| 38. Does your baby communicate with you by using his or her finger to point at objects or pictures? | 5.6 | 12 | 5.8 | 18 | 19.2 | 24 | 69.4 | 46 |
| 39. Do you get the feeling that your baby plays or communicates with you less now than in the past? | 87.8 | 80 | 4.4 | 14 | 1.7 | 5 | 5.3 | 1 |
| 40. Do your baby's eyes line up together when looking at an object? | 5.8 | 1 | 1.5 | 1 | 4.3 | 3 | 85.8 | 95 |
| 41. Are your baby's feeding patterns regular from day to day? | 1.2 | 1 | 1.1 | 2 | 7.3 | 19 | 90 | 78 |
| 42. Does your baby enjoy rubbing or scratching toys or objects for long periods of time? | 40.6 | 49 | 21 | 34 | 22.8 | 13 | 14.9 | 4 |
| 43. Does your baby seem to get his or her body stuck in a position or posture that is hard to move out of? | 77.9 | 70 | 13.7 | 23 | 6.2 | 6 | 1.2 | 1 |
| 44. Does your baby enjoy making objects spin over and over in the same way? | 43.2 | 32 | 21.4 | 33 | 26.3 | 27 | 8.7 | 8 |
| 45. While lying down, does your baby enjoy kicking his or her feet over and over for long periods of time? | 32.1 | 42 | 18.1 | 33 | 30.1 | 19 | 19.2 | 6 |
| 46. Does your baby stare at his or her fingers while wiggling them in front of his or her eyes? | 47.8 | 32 | 17.4 | 35 | 24.4 | 27 | 10 | 6 |
| 47. which of the following best describes your baby's typical play with a favorite toy? | 10.4 | 12 | 30.6 | 55 | 58.3 | 33 | | |
| 48. which of the following describes your baby's interest in toys on a typical day? | 4.7 | 3 | 23.4 | 27 | 71.2 | 70 | | |
| 49. When you introduce your baby to a new game (peek-a-boo, so-big, patty-cake, etc.) how does your baby respond? | 86.6 | 29 | 11.9 | 63 | 0.9 | 6 | 0.2 | 2 |
| 50. What do you typically have to do to get your baby to look up from playing with a favorite toy? | 68.2 | 43 | 25.4 | 54 | 5.9 | 3 | | |
| 51. What is your baby's usual reaction to somewhat painful experiences, like bumping his or her head? | 2.7 | 4 | 89.2 | 93 | 7.5 | 3 | | |
| 52. What do you typically have to do to get your baby to turn towards you? | 88.9 | 71 | 9.3 | 25 | 1.5 | 4 | | |
| 53. What do you typically have to do to get your baby to smile or laugh at you? | 92.1 | 92 | 6.5 | 8 | 0.9 | <1 | | |

**Table 3.** *Cont.*

| | Never | | Seldom | | Sometimes | | Often | |
|---|---|---|---|---|---|---|---|---|
| | **ItS** | **AS** | **ItS** | **AS** | **ItS** | **AS** | **ItS** | **AS** |
| 54. On a typical night, how many hours does your baby sleep? | 4.9 | 13 | 36.7 | 71 | 46.9 | 14 | 11.1 | 2 |
| 55. On a typical night, how many times does your baby wake up? | 27.5 | 51 | 55.1 | 43 | 16.7 | 6 | | |
| 56. which of the following best describes your baby's skill level? | 19.8 | 48 | 44.9 | 44 | 29.5 | 6 | 5.2 | 2 |
| 57. which of the following best describes your baby's typical day? | 76.3 | 28 | 21.2 | 59 | 1.7 | 11 | 0.2 | 2 |
| 58. If you start a game by copying or imitating a sound your baby makes, what does your baby typically do? | 0.6 | <1 | 7 | 11 | 26.9 | 35 | 64.4 | 54 |
| 59. When your baby is awake and not eating, does your baby keep a toy or object in his or her mouth? | 22.1 | 29 | 37.4 | 50 | 30.6 | 17 | 9.4 | 4 |
| 60. which of the following best describes the way your baby coordinates his or her eyes and hands while playing with a toy? | 89.8 | 81 | 7.3 | 19 | 1.5 | <1 | 0.9 | <1 |

Note: IS = Italian Sample; AS = American Sample. The bold line identifies the items with three or four multiple-choice answers.

**Table 4.** Chi-square comparison between Italian (ItS) and Israeli (IS; Ben-Sasson and Carter, 2012) sample response distribution (%).

| | Never | | Seldom | | Sometimes | | Often | |
|---|---|---|---|---|---|---|---|---|
| | **ItS** | **ISS** | **ItS** | **ISS** | **ItS** | **ISS** | **ItS** | **ISS** |
| 3. Does your baby seem overly sensitive to your touch (for example, fuss or pull away when you touch him or her)? | 71.2 | 83 | 19.4 | 14 | 6.2 | 2.1 | 2.4 | 1.3 |
| 6. When you and your baby are facing each other, does your baby turn his or her eyes to avoid looking at you? | 81.7 | 70 | 11.9 | 21 | 4.7 | 7 | 1.1 | 1 |
| 9. Does your baby spit out certain textures of foods, such as lumpy or chunky pieces? | 25.3 | 26 | 18.4 | 38 | 35.3 | 30 | 19.6 | 7 |
| 13. Does your baby rock his or her body back and forth over and over? | 61 | 39 | 14.8 | 25 | 16.7 | 31 | 7 | 10 |
| 17. Does your baby forcefully press his or her face, head, or body against people or furniture? | 79.6 | 59 | 10 | 23 | 7.6 | 15 | 2.1 | 3 |
| 23. When your baby is awake and you pick him or her up, does your baby's body feel loose or floppy? | 87.4 | 26 | 7 | 16 | 3.3 | 28 | 1.4 | 30 |
| 30. Does your baby get stuck doing a simple activity over and over? | 79.3 | 25 | 14.6 | 40 | 4 | 31 | 1.1 | 4 |
| 35. When you say "Where's (a familiar person or object)?" without pointing or showing, will your baby look at the person or object named? | 0.6 | 11 | 2 | 14 | 13.7 | 39 | 83.3 | 36 |
| 37. Does your baby seem to get stuck on playing with a part of a toy (such as an eyeball, label, wheel or tag), instead of the whole toy? | 16.1 | 9 | 18.1 | 20 | 34.1 | 37 | 31.4 | 34 |
| 43. Does your baby seem to get his or her body stuck in a position or posture that is hard to move out of? | 77.9 | 53 | 13.7 | 38 | 6.2 | 8 | 1.2 | 2 |
| 48. which of the following describes your baby's interest in toys on a typical day? | 4.7 | 5 | 23.4 | 41 | 71.2 | 55 | | |
| 55. On a typical night, how many times does your baby wake up? | 27.5 | 20 | 55.1 | 61 | 16.7 | 19 | | |
| 56. which of the following best describes your baby's skill level? | 19.8 | 14 | 44.9 | 54 | 29.5 | 26 | 5.2 | 6 |
| 58. If you start a game by copying or imitating a sound your baby makes, what does your baby typically do? | 0.6 | 0.4 | 7 | 19 | 26.9 | 51 | 64.4 | 30 |

Note: ITS = Italian Sample; IS = Israeli Sample. The bold line identifies the items with three or four multiple-choice answers.

Comparing the Italian and the American distributions, the $\chi^2$ values were below the critical values ($\chi^2_{0.05,3} = 7.815$; $\chi^2_{0.05,2} = 5.991$); thus, no differences emerged between the two samples. Similarly, comparing the Italian and the (partially available) Israeli distribution, no difference emerged.

Score Distributions and Cutoffs for ASD Risk. In Table 5, we summarized our scores and those obtained by the other international studies. It was not always possible to compare Italian findings with those of the American and Israeli studies since some data were not available in the papers.

**Table 5.** Comparison between the American, Israeli and Italian cutoffs.

| | **Reznick et al., (2007) *n* = 1300** | **Ben-Sasson and Carter, (2012) *n* = 471** | **Italian Sample *n* = 657** |
|---|---|---|---|
| **Range** | 0–50 (theoretical range) | 0–33.88 | 0–20.32 |
| **Modal score** | 0 | | 0 |
| **Median score** | 5.75 | 9.13 | 2.74 |
| **Mean score** | - | 10.40 (*sd* = 6.38) | 3.29 (*sd* = 2.74) |
| **Total risk score (≥95th percentile)** | 17.75 * | 22.55 | 17 |
| **Total risk mean score (≥95th percentile)** | - | - | 8.15 |
| **Social–Communication domain score (95th percentile)** | - | 27.85 | 7 |
| **Sensory–Regulatory Functions domain (95th percentile)** | - | 26.95 | 10 |
| **Total risk score (98th percentile)** | 22.62 ** | 28.14 | 21 |
| **Children at risk on 95th percentile** | - | 4.88% | 4.87% |

* Ben-Sasson and Carter reported that this value was from a personal communication by Reznick. ** This value was not reported in Reznick and colleagues (2007), it was calculated according to Ben-Sasson and Carter (2012). "-" Means the values were not reported in the paper.

Nevertheless, it is worth noting that the American data range was only theoretical and that the Israeli data range was higher than that in the Italian results. The modal value was 0 both in the American and the Italian data; this confirmed that, in the general population, the majority of FYI scores strived towards the lowest score that indicated typical development. With regard to the mean score, it was possible to compare Italian and Israeli data: the first score was lower than the second. The American mean score was not available.

Finally, with regards to the cross-cultural risk score comparison, it is worth noting, as few values were reported by the American authors, that the only two values reported in Table 6 were calculated according to Ben-Sasson and Carter's [31] suggestions. The American and Italian data comparison on risk score on the 95th and 98th percentile showed similar values. Figure 2 shows the distribution of risk score (skewness = 1.53; kurtosis = 4.03) for the Italian sample and the shape inflection corresponding to the score of 17, as found in Reznick and colleagues' [8] study. Comparing Israeli and Italian data, the Italian raw values corresponding to the 95th and 98th percentile were lower than the Israeli ones.

**Table 6.** Correlations among the FYI 8 constructs on the Italian sample. Between parentheses, the results of the correlations yielded in the American sample by Reznick and colleagues (2007).

| FYI Construct | Social–Communication Domain | | | Sensory–Regulatory Functions Domain | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| Social Orienting a Receptive Communication | 0.16 *** (0.42 **) | 0.32 *** (0.38 **) | 0.12 *** (0.42 **) | 0.07 (0.19 **) | 0.08 * (0.10 **) | 0.10 * (0.13 **) | 0.13 ** (0.12 **) |
| Social-Affective Engagement (1) | | 0.28 *** (0.33 **) | 0.30 *** (0.49 **) | −0.01 (0.03) | 0.04 (0.04) | 0.08 * (−0.01) | 0.11 ** (0.04) |
| Imitation (2) | | | 0.20 *** (0.35 **) | 0.07 (0.12 **) | 0.09 * (0.03) | 0.13 *** (0.10 **) | 0.09 * (0.02) |
| Expressive Communication (3) | | | | −0.05 (0.07) | 0.05 (0.03) | 0.06 (0.03) | −0.01 (0.04) |
| Sensory Processing (4) | | | | | 0.14 *** (0.18 **) | 0.13 ** (0.30 **) | 0.34 *** (0.38 **) |
| Regulatory Pattern (5) | | | | | | 0.08 * (0.15 **) | 0.06 (0.11 **) |
| Reactivity (6) | | | | | | | 0.14 *** (0.10 **) |
| Repetitive Behavior (7) | | | | | | | |

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; df: 655.

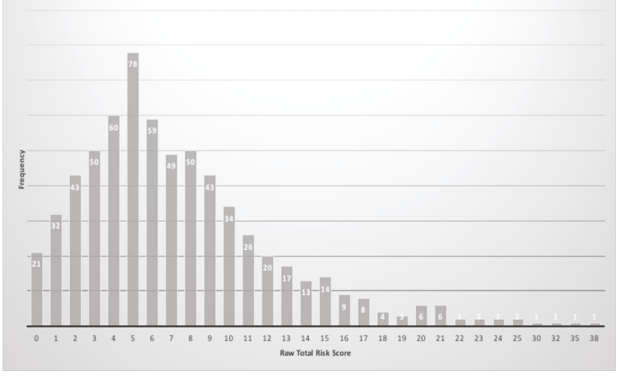


**Figure 2.** Distribution of First Year Inventory (FYI) raw risk scores in the Italian sample according to the factor structure of Reznick et al. (2007).

According to the other two validation studies on a general population [8,31], scores equal or above the 95th percentile could be applied to detect children at risk for ASD. We decided to apply the mean score on the 95th percentile of the total score, which was 8.15; 32 children in our sample met this risk criterion (which corresponds to 4.87% of the sample). a similar result (4.88%) was found by Ben-Sasson and Carter [31] on the Israeli general population. The families with children under the risk condition were invited for a diagnostic assessment with gold standard measures. The evaluation is in progress, and the children have been followed over time.

### *3.3. Construct Validity*

To investigate the inter-correlations between the two domains and the eight constructs, Pearson r correlations were carried out. Table 6 reports the correlations between the eight constructs and also the correlations found in Reznick and colleagues' [8] study as a comparison. As expected, the four

constructs of the Social–Communication domain correlated with each other, as did the four constructs of the Sensory–Regulatory Functions domain. Furthermore, results showed that the Expressive Communication construct (part of the Social–Communication domain) did not correlate with all constructs of the Sensory–Regulatory Functions domain; Social–Affective Engagement (part of the Social–Communication domain) did not correlate only with the Sensory Processing and Regulatory Pattern constructs (part of the Sensory–Regulatory Functions domain). The two domains are correlated as well, r = 0.13, $p = 0.01$.

### *3.4. Internal Consistency and Factorial Analyses*

The Hayes and Krippendorff' kalpha for Social–Communication and Sensory–Regulatory Functions domains were 0.91 and 0.88, respectively. These values were higher than those found by Reznick and colleagues [8] and suggested a moderate consistency among items.

Exploratory factor analyses (EFA) and confirmatory factor analyses (CFA) through SEM (Structural Equation Modelling) were carried out in Mplus v.8 applying WLSMV because the data were ordinal. Geomin rotation was applied to the EFAs. a first-order CFA was performed on the 52 items of the FYI to test the eight-factor structure corresponding to the constructs hypothesised by Reznick and colleagues [8] (Figure 3). The 10 items that did not load for any factor (see Appendix in Reznick et al.'s paper, [8]) and were not inserted into the analysis. a second-order CFA was tested based on the second-order factorial structure estimating the eight constructs (as first level latent factors) and the two domains (as second-level latent factors). For both CFAs, values of the $\chi^2$, the CFI (Comparative Fit Index), and the RMSEA (Root Mean Square Error of Approximation) were examined. The two CFAs showed several correlations between items or between constructs with values close or equal to 1, suggesting that the items or the factors should be collapsed.

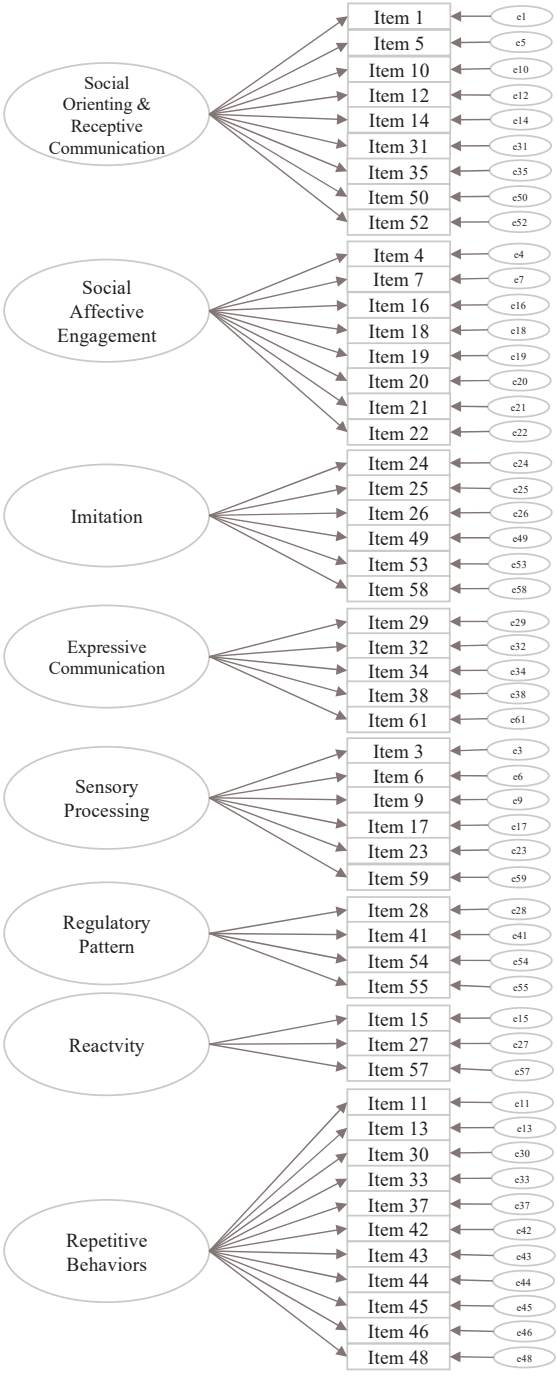


**Figure 3.** A graphical reproduction of FYI Factor Structure by Reznick et al. (2007).

As both the CFAs failed to estimate acceptable factorial structures, we chose to go back to the EFA. Two factorial structures were tested on the original 61 items: the eight-factor structure, corresponding to the eight constructs, and the two-factor structure, corresponding to the Socio-Communication domain and the Sensory–Regulatory Functions domain. The $\chi^2$, the CFI, and the RMSEA were examined for both. The items were progressively excluded if the factor loadings loaded for two or more factors or none of them. The comparison between the eight-factor and the two-factor structure showed that the latter was the best fitted. Thus, we performed a further test via CFA.

The first order CFA on the 52 items yielded a moderate–low fit of the data, with a significant $\chi^2$ (1196) = 2214.53, $p < 0.001$, and CFI = 0.83, RMSEA = 0.036 (LO90% = 0.034, HI90% = 0.038). Similarly, the second order CFA showed moderate-low fit of the data, with a significant $\chi^2$ (1214) = 2238.99, $p < 0.001$, and CFI = 0.83, RMSEA = 0.036 (LO90% = 0.034, HI90% = 0.038).

The EFA on the 61 items estimating the eight-factor structure was well fitted, $\chi^2$ (1318) = 1585.34, $p < 0.001$, CFI = 0.96, RMSEA = 0.018 (LO90% = 0.014, HI90% = 0.021). However, 43 items were excluded because the factor loadings loaded for two or more factors and the remaining items loaded for four factors instead of the hypothesised eight, and those four factors did not correspond with the theoretical model hypothesised by Reznick and colleagues [8].

For these reasons, the EFA estimating the two-factor structure was preferred and reached a moderate fit of the data, $\chi^2$ (1651) = 2940.15, $p < 0.001$, CFI = 0.79, RMSEA = 0.034 (LO90% = 0.032, HI90% = 0.036). Nineteen items (Items 4, 5, 6, 16, 27–29, 31, 32, 39, 41, 49–56) were excluded from the subsequent analysis because the factor loadings loaded for two or more factors. After exclusion of those items, the subsequent fourth EFA reached moderate fit of the data, $\chi^2$ (739) = 1185.47, $p < 0.001$, CFI = 0.92, RMSEA = 0.03 (LO90% = 0.027, HI90% = 0.033). Items 7, 14, 44, and 48 did not load for any factor and were subsequently deleted. The third EFA reached moderate fit of the data, $\chi^2$ (593) = 996.58, $p < 0.001$, CFI = 0.92, RMSEA = 0.03 (LO90% = 0.029, HI90% = 0.036), and again, Items 11 and 57 did not load for any factor and were subsequently deleted. a final EFA was carried out with the remaining items (Factor 1: $n = 15$ items; Factor 2: $n = 16$ items), again showing moderate fit of the data, $\chi^2$ (526) = 921.79, $p < 0.001$, CFI = 0.92, RMSEA = 0.034 (LO90% = 0.030, HI90% = 0.037). Table 7 shows the final EFA solution. Factor 1 contains items corresponding to the Social–Communication Domain, Factor 2 to the Sensory–Regulatory Functions Domain, so all the items loaded for the expected factor.

**Table 7.** Exploratory Factor Analysis (EFA) results (standard errors between parentheses).

| | 1 | 2 |
|---|---|---|
| FYI_1 | **0.544 (0.091)** | −0.196 |
| FYI_10 | **0.550 (0.051)** | −0.092 |
| FYI_12 | **0.345 (0.055)** | −0.025 |
| FYI_18 | **0.304 (0.082)** | −0.045 |
| FYI_19 | **0.655 (0.039)** | −0.159 |
| FYI_20 | **0.708 (0.036)** | −0.131 |
| FYI_21 | **0.625 (0.047)** | −0.168 |
| FYI_22 | **0.570 (0.038)** | −0.005 |
| FYI_24 | **0.653 (0.037)** | −0.046 |
| FYI_25 | **0.851 (0.031)** | −0.003 |
| FYI_26 | **0.787 (0.034)** | −0.004 |
| FYI_34 | **0.522 (0.080)** | −0.047 |
| FYI_35 | **0.578 (0.049)** | −0.160 |
| FYI_38 | **0.642 (0.036)** | −0.114 |
| FYI_58 | **0.425 (0.047)** | −0.040 |
| FYI_2 | 0.017 | **0.239 (0.044)** |
| FYI_3 | −0.037 | **0.335 (0.051)** |
| FYI_8 | −0.061 | **0.258 (0.048)** |
| FYI_9 | 0.054 | **0.198 (0.046)** |
| FYI_13 | −0.106 | **0.651 (0.035)** |
| FYI_15 | −0.087 | **0.337 (0.041)** |

**Table 7.** *Cont.*

| | 1 | 2 |
|---|---|---|
| FYI_17 | 0.003 | **0.434 (0.052)** |
| FYI_23 | −0.149 | **0.583 (0.059)** |
| FYI_30 | −0.049 | **0.774 (0.036)** |
| FYI_33 | −0.158 | **0.729 (0.029)** |
| FYI_37 | −0.112 | **0.607 (0.032)** |
| FYI_42 | −0.048 | **0.701 (0.027)** |
| FYI_43 | −0.080 | **0.682 (0.039)** |
| FYI_45 | −0.072 | **0.695 (0.028)** |
| FYI_46 | −0.075 | **0.693 (0.028)** |
| FYI_59 | −0.034 | **0.343 (0.040)** |

The final EFA structure was tested via CFA. The two-factor structure showed moderate fit of the data, $\chi^2$ (433) = 672.72, $p < 0.001$, CFI = 0.95, RMSEA = 0.029 (LO90% = 0.026, HI90% = 0.033). Item 9 had low factor loading with the factor and was subsequently deleted. The two factors were weakly correlated, r = 0.15, $p = 0.045$. The final CFA was carried out showing good fit of the data, $\chi^2$ (404) = 617.699, $p < 0.0001$, CFI = 0.95, RMSEA = 0.028 (LO90% = 0.024, HI90% = 0.033). Figure 4 shows the factor structure obtained by the CFA.

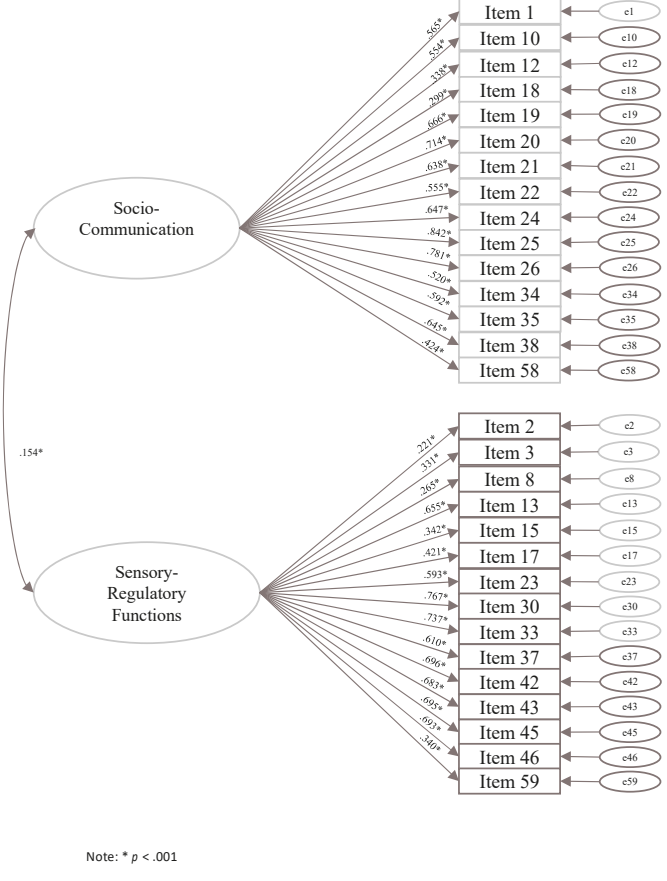


**Figure 4.** FYI Structure according to Confirmatory Factor Analysis (CFA) run in this study.

After those analyses, we re-examined our data according to the new factorial structure. The total score ranged from 0 to 18.39, with a mean of 3.27 (SD = 3.04), a median of 2.17, and a distribution shaped as a chi-square (skewness = 1.49; kurtosis = 2.86). The t-tests showed a significant difference by children's gender, $t(648) = 2.062$, $p = 0.040$, with boys reaching a higher total score (M = 3.48; ds = 3.06) than the girls (M = 2.99; ds = 2.95). There were no significant differences by childbirth order or by parents' marital status. In contrast, the t-tests showed significant differences by educational level on the Sensory–Regulatory Functions domain, $t(208.185) = 3.537$, $p < 0.0001$ and on the total score, $t(206.433) = 3.755$, $p < 0.0001$. Specifically, mothers with a low educational level showed higher scores (Sensory–Regulatory Functions domain: M = 6.70; ds = 5.98; total score: M = 4.18; ds = 3.51) than mothers with a high educational level (Sensory–Regulatory Functions domain: M = 4.79; ds = 4.87; total score: M = 2.99; ds = 2.82). Finally, the risk cutoff on the 95th percentile of the total score corresponded to a score of 9.14.

## 4. Discussion

The main purpose of this study was to conduct an early screening of the signs of risk of ASD, applying the FYI as part of pediatric surveillance on an Italian sample from the general population. We examined the cross-cultural generalisability of the screening measure, comparing the Italian scores with those of the two validation studies conducted on a general population [8,31]. The other two aims of the research were to test the construct validity of the FYI and to demonstrate its internal consistency and structural validity.

The combination of all the results mentioned represents a demonstration of the generalisability and stability of the measure across cultures. First of all, we considered the role played by the socio-demographic variables and compared the present findings with those found with the American and Israeli samples. Significant differences were found by children's gender, with boys showing higher scores than girls for the Reactivity construct (part of the Sensory–Regulatory Functions domain).

Considering the parental variables, it is worth noting that in the other two validation studies on the general population [8,31], among the socio-demographic variables considered, the authors examined whether maternal ethnicity influenced the scores of the FYI (Reznick et al., 200). Those differences were not tested on the Italian sample, because all the parents were European–White. Reznick and colleagues [8] and Ben-Sasson and Carter [31] also considered the educational level and marital status of the mothers.

Similarities between the Italian and American and Israel samples were also found for the maternal educational level and marital status. As Reznick and colleagues [8] and Ben-Sasson and Carter [31] found, a low educational level was associated with higher FYI scores compared to a high educational level. One possible explanation is that mothers with a low educational level may interpret several atypical behaviours as common because they misunderstood the meaning of the item [31]. In particular, the items of the Sensory–Regulatory Functions domain describe atypical behaviours, as they would be 'positive' (i.e., presence of a behaviour) instead of 'negative' (i.e., absence of a developmentally expected behaviour). For example, the item, 'Is your baby content to play alone for an hour or more at a time?' can be misleading because the mothers may interpret as positive the fact that child plays quietly alone for long periods (i.e., presence of a behaviour).

Moreover, we found that single mothers reported higher FYI scores on the Repetitive Behaviors construct (part of the Sensory–Regulatory Functions domain) than did married mothers. Ben-Sasson and Carter [31] found a similar result for the Sensory–Regulatory Functions domain. The explanations of these results may be twofold. First, the single mothers did not have a partner with whom they could discuss concerns about the child's development; thus, they could interpret the child's Sensory–Regulatory behaviours as atypically. Second, the child's self-regulation process may be affected by the absence of the father [38].

As a further demonstration of the cross-cultural generalisability of the FYI, we found similar patterns of response for each item, meaning that there were no differences across cultures in the way in

which parents of children from 11 to 13 months of age replied to the questions. This result highlighted that targeted behaviours evaluated by the FYI were identifiable in a similar manner across different cultures. Thus, this property allows the detection of typical and atypical behaviours that appear to be cross-culturally invariant.

Finally, the Italian results were similar to the American findings for the total risk score calculated on the 95th and 98th percentile, and both were lower than the risk scores calculated on the Israeli sample. As Ben-Sasson and Carter [31] suggested, this could be due to the dysregulation [39] and the stress [40] endured by Israeli children growing-up in a stressed society faced with trauma and terror daily.

Nevertheless, the percentage (32%) of children detected at risk (with a total score equal or above the 95th percentile) in the Italian and the Israeli samples was similar (these data were not available in Reznick and colleagues' [8] study).

The second aim examined the FYI construct validity. The positive and significant correlations between the two domains of the instrument (Social–Communication and Sensory–Regulatory Functions) and between constructs highlighted a good construct validity of the measure, as found by Reznick and colleagues [8].

Since no previous studies on the FYI have validated its factorial structure, the purpose of the present study was to give insight on this property. In this vein, the Confirmatory Factor Analysis is a crucial and strategical analysis demonstrating the structural validity of a measure. Therefore, we firstly carried out a CFA on the theoretical structure hypothesized by Reznick and colleagues [8]. Our analyses did not confirm the structure of the scale organised on the eight hypothesised constructs. It should be noticed that Reznick and colleagues [8] also struggled to find a stable factorial solution for their data and decided to shape the final eight constructs through the item–total correlations and the expected thematic content of the items. As the second step in our study, two second-order latent factors, corresponding to the two main domains of the FYI, were estimated through CFA. Even in this case, the results did not support the hypothesised structure. Therefore, we decided to explore the structure of our data with a set of five nested EFAs in which several items were found as critical, because of loading more than one factor or because of not showing the expected factor loading (i.e., $> 0.30$), and deleted step by step. The final explorative factorial structure comprehended 30 items, which are coherently distributed in the Social Communication and Sensory–Regulatory Functions domains. a CFA confirmed this structure and allowed the estimation of a short version of the FYI, which was suggested by Turner-Brown and colleagues [34] as one point to be developed by future research after their study. The short version of the questionnaire makes its administration easier and faster and allows applying the questionnaire during systematic screening evaluations on the general population.

The short version of the FYI evaluated the two main core areas of risk for ASD, in which the main symptoms are included, as suggested by Reznick and colleagues [8] and the DSM-5 [1]. Most of the items of the short version assess the social and communicative deficit (Factor 1), focusing on the evaluation of receptive communication and social engagement. The others evaluate the first factor focussing on child's imitative capacity, and his/her expressive communication. Furthermore, the second factor estimated in the short version (Sensory–Regulatory Functions domain) evaluates the presence of repetitive behaviours and the hypo- or hypersensitivity of the child to sensory stimuli. The evidence on the FYI short version highlighted the expected results considering both the parental and the children's socio–demo variables, as found in the other validation studies [8,32] who applied the full version of FYI.

The total score calculated on the final structure of the scale showed significant difference by gender, with boys reaching higher total scores compared to the girls, confirming the American and Israeli findings and the gender ratio of ASD (4:1) [1]. Even with the total score calculated on the short version (FYI-30), low parental educational level was associated with higher total score compared to the opposite condition, whereas marital status was not significant. Therefore, the estimated short version seems to represent the two core symptoms of the ASD and, at the same time, maintains the impact of the socio-demographic variables on the total score as found by previous research.

## 5. Limitation

The main limitation of the present study is the cross-sectional design. Longitudinal studies on the general population are required to demonstrate the accuracy of the FYI, its PPV (i.e., positive predictive value) and NPV (i.e., negative predictive value), and ability to detect signs of risk of ASD. Future studies, starting from our results on the FYI short version, should consider the diagnostic outcome evaluation, through gold-standard measures, and the convergent validity. Specifically, the evaluation should be focused on the severity of the autistic traits, the global child development, and characteristics of attention-selectivity processes [41]. a prospective study is currently ongoing with a longitudinal evaluation of children considered to be at risk at 11–13 months of life and evaluated one and two years later. Furthermore, other studies should further demonstrate the short version structure of the FYI developed in this study. The second limitation is related to the relatively low response rate of the professionals in our study, although it is similar to what was found by others [8,32]. It should be noticed that the low response rate of the professionals did not correspond to a similar low parental response rate. Indeed, when the paediatrician participation was obtained, on their side, parents easily agreed to be participants. It is highly likely that parental participation depends on the quality of their relationship with the paediatrician, as found by others [42,43]. This also means that a way to establish a continuous screening for children's mental health and speed up early diagnosis and intervention is increasing health professionals' awareness of that aspect.

## 6. Conclusions and Implication

According to our results, the FYI is a valid and reliable screening tool for Italian children. Results for the current study stimulate further research in the field of cross-cultural validity and generalisability of the FYI and other measures for the early identification of signs of risk of ASD.

Our findings highlighted some positive features of the FYI and, at the same time, several others that should be further developed. On the one hand, the analyses have shown the cross-cultural stability and generalisability of the FYI as well as its construct validity. Therefore, the FYI is a reliable tool that may be administered in another cultural context from the American and Israeli ones.

On the other hand, modest demonstrations of internal consistency were found, as also confirmed by the factorial analyses. As for the latter, the hypothesised structure (see [8] for details) did not receive appropriate support, showing poor fit of the data with several correlations between items with values close or equal to 1. The alternative analyses carried out revealed a structure organised on the two main core symptoms of ASD, also identified by Reznick and colleagues [8] in their original version of the FYI, based on a short version of the questionnaire. Our analyses demonstrated that the factorial validity of the FYI requires further demonstration. This notwithstanding, the short version of the FYI may lead to a cost-effective and easy-to-administer instrument to be used by paediatricians during their pediatric surveillance on the general population. The early detection of atypical developmental trajectories may support medical decision-making on further steps for referral of the child to an early diagnostic assessment (which may enable early intervention when needed; [43–48].

**Author Contributions:** Conceptualization, F.L. and S.P.; methodology, A.L., F.L., and S.P.; software, A.L. and S.P.; formal analysis, A.L. and S.P.; investigation, A.L.; data curation, A.L. and S.P.; writing—original draft preparation, A.L., F.L., and S.P.; supervision, F.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Acknowledgments:** We are grateful to the two associations of paediatricians, the Italian Federation of Medical Pediatricians (FIMP) and the Italian Confederation of Pediatrics (CIPe), for their collaboration. We are also very grateful to all paediatricians and parents for their participation. We are grateful to the psychologists Filomena De Lumè, Luigia Duma, Valentina Garrapa, Emilia Perrone, Viviana Vetrugno, Annamaria Vizzi, Giuseppe Antonioli for their collaboration for data collection.

**Conflicts of Interest:** The authors declare no conflict of interest.

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# Inflammatory Biomarkers are Correlated with Some Forms of Regressive Autism Spectrum Disorder

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Received: 12 November 2019; Accepted: 9 December 2019; Published: 11 December 2019

**Abstract:** *Background*: Several studies have tried to investigate the role of inflammatory biomarkers in Autism Spectrum Disorder (ASD), and their correlations with clinical phenotypes. Despite the growing research in this topic, existing data are mostly contradictory. *Methods*: Eighty-five ASD preschoolers were assessed for developmental level, adaptive functioning, gastrointestinal (GI), socio-communicative and psychopathological symptoms. Plasma levels of leptin, resistin, plasminogen activator inhibitor-1 (PAI-1), macrophage chemoattractant protein-1 (CCL2), tumor necrosis factor-alfa (TNF-α), and interleukin-6 (IL-6) were correlated with clinical scores and were compared among different ASD subgroups according to the presence or absence of: (i) GI symptoms, (ii) regressive onset of autism. *Results*: Proinflammatory cytokines (TNF-α, IL-6 and CCL2) were lower than those reported in previous studies in children with systemic inflammatory conditions. GI symptoms were not correlated with levels of inflammatory biomarkers except for resistin that was lower in ASD-GI children ($p = 0.032$). Resistin and PAI-1 levels were significantly higher in the group with "regression plus a developmental delay" onset (Reg+DD group) compared to groups without regression or with regression without a developmental delay ($p < 0.01$ for all). *Conclusions*: Our results did not highlight the presence of any systemic inflammatory state in ASD subjects neither disentangling children with/without GI symptoms. The Reg + DD group significantly differed from others in some plasmatic values, but these differences failed to discriminate the subgroups as possible distinct ASD endo-phenotypes.

**Keywords:** autism spectrum disorder; regression; cytokines; PAI-1; neuroinflammation; gastrointestinal

## 1. Introduction

To date, the understanding of the underlying molecular mechanisms of some metabolic or neurological diseases and the deepening of knowledge on the role of inflammation in these disorders have radically changed our understanding of their etiology [1,2]. Alzheimer's (AD) and Parkinson's disease, type 1 and type 2 diabetes, and obesity are just some of the pathologies for which a well-defined

role of inflammation has been identified, with consequent possible therapeutic implications [3,4]. For example, activated astrocytes and microglia are characteristically found in abundance near neurons and plaques in AD [5] and the block of the activation of insulin signaling receptors caused by the chronic exposure of pro-inflammatory mediators in β-cells of pancreatic islets has been evidenced in the pathogenesis of insulin resistance which underlies many metabolic diseases [6,7].

Recently, the contribution of immune dysregulation has been described as a common feature of the autism spectrum disorder (ASD), and alterations in circulating cytokine levels have been repeatedly reported [8,9]. ASD are neurodevelopmental disorders characterized by persistent social communication difficulties with concurrent restricted interests, repetitive activities and sensory abnormalities [10]. The etiopathogenesis of idiopathic ASD is complex and not yet fully elucidated, but it is widely recognized that genetic liability and environmental factors interact in producing early alteration of structural and functional brain development, responsible for ASD symptoms [11,12]. Despite a systematic review about proinflammatory markers in more than 3900 children and/or adolescents with neuropsychiatric disorders including ASD [13] found preliminary evidence for the role of inflammation and pro-inflammatory state in these conditions, until now conflicting and irreproducible findings have been detected in various studies.

Some authors have proposed interleukin (IL)-6, tumor necrosis factor-alpha (TNF)-α, and macrophage chemoattractant protein-1 (CCL2) as potentially involved in brain inflammation at least in a subgroup of subjects with ASD [14]. A recent meta-analysis of 25 studies revealed a higher concentration of pro-inflammatory cytokines interferon (IFN)-γ, IL-1β, IL-6, and TNF-α in children with ASD compared with controls [9]. Increased levels of IL-6 and IL-8 were found to be predictive biomarkers for ASD risk in a study analyzing circulating cytokine patterns from neonatal blood [15]. High levels of IL-6 in the brain could determine alterations of synapse formation, dendritic spine development, and neuronal circuit balance [16], while in plasma they have been associated with increased stereotypical behaviors and with regressive forms of ASD [17]. Conversely, TNF-α has a critical role in regulating synaptic strength and plasticity [18], and his levels have been positively correlated with ASD severity [19]. High CCL2 levels could be instead considered as a signal of microglia/astroglia activation [20], and have been associated with higher aberrant behavior scores and more impaired adaptive functioning [21].

Similarly, GI problems that frequently occur in ASD subjects seem to be caused by inappropriate immune activation and pro-inflammatory processes of the digestive tract [22]. It has been shown that the level of stress-responsive cytokines, like IL-6 and TNF-α, are increased both in ASD subjects [17] and in the general population in association to gastrointestinal (GI) symptoms [23,24], pointing to a link between peripheral inflammation and neuroinflammation. Particularly, high levels of TNF-α can influence the intestinal epithelial barrier possibly contributing to GI problems [25] and intestinal permeability, and also to ASD onset as recently suggests by the "leaky gut" hypothesis [26]. The myeloid dendritic cells, which produce among others TNF- α and IL-6, have been associated with increased GI symptoms in ASD as well as increased amygdalar volume and regressive autism [27]. More recently, other authors [22,28] did not confirm an association between the symptoms of the lower GI tract and levels of TNF-α or IL-6, however their levels were correlated with irritability, socialization and intelligence in ASD subjects.

Besides, a particular type of cytokines called adipokines seems to be implicated in the pathogenesis of inflammatory central nervous system (CNS) disorders and ASD [29] despite the findings obtained so far are mostly controversial. Adipokines, or adipocytokines, are active proteins secreted by white adipose tissue with functions similar to hormones in inter-organ communication [30] and their dysregulation has been implicated in obesity, type 2 diabetes, cardiovascular disease and recently, in peripheral tissue insulin resistance and inflammation [31]. Leptin, adiponectin and resistin are the only three molecules that belong exclusively to the class of adipokines and they have been studied in a limited number of researches concerning autism. Increased levels of leptin, decreased levels of resistin and a negative correlation between the levels of adiponectin and the severity of social impairment were

found in the plasma of ASD subjects vs. controls [29]. Previously, Blardi et al. [32,33] found higher levels of leptin in patients with Rett syndrome in comparison with healthy female subjects, as reported by Ashwood et al. [34] in patients with autism compared to typically developing controls. Leptin dysregulation has been proposed as a mechanism of psychopathology associated with mental health disorders [35], and elevated circulating leptin was consistently found in childhood neurodevelopmental disorders including ASD [34].

Resistin has been implicated in the pathogenesis of several inflammatory CNS disorders [36] and its levels are related to immune changes in autistic subjects: it has been shown that proinflammatory cytokines may increase the expression of messenger-RNA resistin [37] with a positive correlation between increasing resistin levels and inflammatory serum cytokines [38]. A recent case-control study [39] found that resistin levels were increased in ASD subjects compared to healthy controls. To date, no studies have investigated differences in adipokines' levels in ASD subjects with or without GI symptoms.

Distally regulated by some cytokines (i.e., IL-6, IL-1, and TNF-α), the plasminogen activator inhibitor-1 (PAI-1) seems to directly influence brain functions causing a neuronal dis-connectivity due to abnormal neuronal migration [40]. PAI-1 may regulate microglial migration and phagocytosis in an autocrine or paracrine manner playing an important role in the regulation of brain microglial activities in health and disease [41]. Moreover, his locus in human maps very close to or within a region in chromosome 7 linked to autism. No association was found between the presence of ASD and a particular polymorphism of the PAI-1 gene promoter that affects the PAI-1 plasma levels [40].

This pilot study aims (i) to investigate the plasmatic levels of several proinflammatory molecules (TNF-α, IL-6, CCL2, leptin, resistin, and PAI-1) in preschoolers with ASD; (ii) to explore the correlation between their plasmatic levels and behavioral profiles in preschoolers with ASD to detect possible specific subgroups within the ASD heterogeneity.

## 2. Materials and Methods

### *2.1. Participants*

A total of 85 ASD preschoolers were included in the study and recruited from November 2015 to February 2018 at the ASD Unit of the IRCCS Stella Maris Foundation (Pisa, Italy), a tertiary care university hospital during a clinical trial on the efficacy of probiotic supplementation in ASD preschoolers [42]. In the present study baseline clinical and biochemical data of recruited subjects were investigated.

ASD diagnosis was performed according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 [10] criteria by a multidisciplinary team. Exclusion criteria were brain anomalies; neurological syndromes/focal neurological signs; anamnesis of birth asphyxia, severe premature birth/perinatal injuries; epilepsy; significant sensory impairment; diagnosis of organic GI disorder or coeliac disease; special diets; recent any-known infections that could influence circulating cytokines.

All children had a comprehensive evaluation including Autism Diagnostic Observation Schedule-2 (ADOS-2) [43], Griffiths Mental Development Scales-Extended Revised (GMDS-ER) [44], Vineland Adaptive Behavior Scales-Second edition (VABS-II) [45], Child Behavior CheckList 1.5-5 (CBCL 1.5-5) [46], Repetitive Behavior Scale-Revised (RBS-R) [47], Social Communication Questionnaire (SCQ) [48]. The "Overall Level of Non-Echoed Spoken Language" item (A1 score) of the ADOS-2 was used to differentiate non-verbal (those with absent language or less than 5 words) from verbal children: 39 participants (46%) were verbal and 46 (54%) were non-verbal. Information about pharmacological treatments and food supplements in the previous 3 months were collected: parents reported an acute or episodic administration of antibiotics (28.2%), probiotics (8.2%), NSAIDs or paracetamol (14.1%), steroids (8.2%), other drugs without effects on GI symptoms (36.5%), and a chronic administration of osmotic laxatives (12.9%). None of the enrolled subjects used psychotropic drugs.

The demographic and clinical characteristics and a complete description of the tools of all participants and in no-verbal vs. verbal groups are reported in Table 1.

**Table 1.** Clinical characteristics of the total sample and in non-verbal vs. verbal group.

| | **Total Sample (*n* = 85; 100%)** | **Non-Verbal (*n* = 46; 54%)** | **Verbal (*n* = 39; 46%)** | ***p*** | ***p*, Age-adjusted** |
|---|---|---|---|---|---|
| **AGE (years) mean ± SD** | 4.14 ± 1.08 (range 2.18–6.11) | 3.74 ± 0.96 | 4.62 ± 1.02 | <0.0001 | - |
| **MALES** | 71 (83.5%) | 38 (44.7%) | 33 (38.8%) | NS | - |
| **FEMALES** | 14 (16.5%) | 8 (9.4%) | 6 (7.1%) | | - |
| **Weight (Kg)** | 17.70 ± 3.09 | 17.06 ± 3.1 | 18.56 ± 2.89 | 0.026 | NS |
| **BMI (Kg/m$^2$)** | 15.95 ± 1.66 (range 12.75–21.43) | 16.07 ± 1.74 | 15.82 ± 1.54 | NS | NS |
| **Head Circumference (cm)** | 51.21 ± 1.69 (range 55–46) | 51.31 ± 1.83 | 51.09 ± 1.54 | NS | NS |
| **ADOS-2 CSS Score [a] (mean ± SD)** | | | | | |
| Social Affect | 6.43 ± 2.05 | 7.06 ± 1.73 | 5.74 ± 2.09 | 0.002 | n.a.* |
| Restricted and Repetitive Behaviors | 8.23 ± 1.46 | 8.56 ± 1.36 | 7.95 ± 1.50 | NS | n.a.* |
| Total | 7.05 ± 1.85 | 7.72 ± 1.50 | 6.41 ± 1.90 | 0.0007 | n.a.* |
| **GMDS-ER [b] (mean ± SD)** | | | | | |
| Performance Quotients | 70.75 ± 23.33 | 61.47 ± 19.42 | 78.75 ± 23.73 | 0.0018 | n.a.* |
| **VABS-II [c] (mean ± SD) Quotients** | | | | | |
| Communication | 50.86 ± 17.79 | 40.76 ± 10.24 | 63.69 ± 17.43 | <0.0001 | n.a.* |
| Daily Living | 66.56 ± 17.07 | 60.46 ± 13.14 | 73.13 ± 18.16 | 0.0002 | n.a.* |
| Socialization | 63.55 ± 15.02 | 57.35 ± 10.36 | 71.15 ± 16.53 | <0.0001 | n.a.* |
| Motor Skills | 71.88 ± 17.85 | 70.89 ± 17.64 | 75.46 ± 16.75 | NS | n.a.* |
| Composite Score | 59.40 ± 19.53 | 52.96 ± 17.52 | 67.23 ± 19.61 | 0.0007 | n.a.* |
| **CBCL 1.5-5 [d] T-score (mean ± SD)** | | | | | |
| Internalizing Problems | 63.85 ± 9.06 | 64.98 ± 8.30 | 62.72 ± 9.64 | NS | NS |
| Externalizing Problems | 57.10 ± 9.09 | 56.71 ± 8.68 | 57.20 ± 9.55 | NS | NS |
| Total Problems | 62.28 ± 10.51 | 62.73 ± 10.68 | 61.69 ± 10.24 | NS | NS |
| Sleep Problems | 58.21 ± 9.11 | 59.62 ± 10.45 | 56.44 ± 6.83 | NS | NS |
| Attention Problems | 64.15 ± 8.21 | 64.66 ± 8.47 | 63.56 ± 7.98 | NS | NS |
| Aggressive Behavior | 56.58 ± 7.13 | 56.27 ± 5.93 | 56.95 ± 8.38 | NS | NS |
| Attention Deficit/Hyperactivity Problems | 59.31 ± 7.70 | 59.58 ± 7.51 | 59.00 ± 8.00 | NS | NS |
| **RBS-R [e] (mean ± SD)** | | | | | |
| Total Score | 19.87 ± 13.87 | 17.67 ± 10.25 | 22.41 ± 16.91 | NS | NS |
| Total Endorsed Score | 12.76 ± 7.27 | 11.91 ± 5.88 | 13.74 ± 8.58 | NS | NS |
| Low Index | 9.44 ± 6.07 | 9.33 ± 5.67 | 9.56 ± 5.59 | NS | NS |
| High Index | 10.25 ± 9.91 | 8.09 ± 7.11 | 12.79 ± 12.04 | 0.028 | 0.028 |
| **SCQ [f] (mean ± SD)** | | | | | |
| Total Score | 14.98 ± 5.90 | 16.72 ± 5.28 | 13.18 ± 6.16 | 0.006 | NS |

[a] ADOS-2 is a semi-structured assessment of communication, social interactions, play, imagination, and stereotyped or repetitive behaviors used as the gold standard tool for the diagnosis of ASD. Higher ADOS-2 CCS scores indicate greater severity of autism (range of possible scores for Total, Social Affect and Restricted and Repetitive Behavior is 1–10). [b] GMDS-ER are a developmental assessment procedure including five different subscales. We used the Performance subscale to measure the non-verbal skills of each child. Higher scores indicate greater non-verbal abilities. Scores around 100 indicate normal non-verbal skills; scores below 70 indicate a developmental delay of non-verbal skills. [c] VABS-II is a parent interview that assesses adaptive functioning in different daily skills. Higher scores indicate greater adaptive skills, scores around 100 indicate a normal adaptive functioning and scores below 70 indicate a delay with respect to age. [d] CBCL 1.5-5 is a parent-report questionnaire that includes 100 statements about the child's behaviors summarized into three summary scales (Internalizing, Externalizing and Total Problems). Besides, we have used the Aggressive Behavior, the Sleep Problems, the Attention Problems and the Attention Deficit/Hyperactivity (ADHD) Problems Scales of this tool as suggested by previous works on this argument. A T-score of 64 and above for summary scales, and a T-score of 70 and above for the other scales, are generally considered clinically significant. Values between 60 and 63 for summary scales, or between 65 and 69 for the other scales, identify a borderline clinical range. [e] RBS-R is a questionnaire completed by parents about the presence of a broad spectrum of repetitive behaviors. Higher scores indicate greater severity (range 0–114). A two-factor solution scoring of RBS-R was also adopted for this study: a Low-Level Index (composed of items pertaining to Stereotyped, and Self-Injurious subscales) and a High-Level Index (composed of items related to Compulsive, Ritualistic, Sameness and Restricted Interests Behaviors subscales). [f] SCQ is a 40-item parent-report screening measure evaluating the symptoms associated with ASD. We used the form "last three months", completed by parents concerning the child's last three months of life. Higher scores indicate greater severity (range 0–39) with a threshold of 15 compatible for a relevant impairment of social communication (some studies consider 9 in children younger than four years old). * Age adjustment is not due for ADOS-2 CCS, GMDS-ER and VABS-II since they are already standardized to compare subjects with different chronological ages. Abbreviations (alphabetic order): ADOS-2 Autism Diagnostic Observation Schedule-2; BMI Body Mass Index; CBCL 1.5-5 Child Behavior Checklist 1.5-5; CSS Calibrated Severity Score; GMDS-ER Griffiths Mental Development Scales-Extended Revised; n.a. not applicable; NS not significant; RBS-R Repetitive Behaviors Scale Revised; SCQ Social Communication Questionnaire; SD standard deviation; VABS-II Vineland Adaptive Behavior Scales-II.

To evaluate the presence of GI symptoms we used a modified version of the GI Severity Index (GSI [49]) splitting the subjects into two groups (GI vs. No-GI). GSI is a score designed to identify signs and symptoms of GI distress commonly reported by parents of children with ASD including nine variables, the first six exploring specific GI symptoms (constipation, diarrhea, stool consistency, stool smell, flatulence, abdominal pain) and three exploring unexplained daytime irritability, night-time awakening, and abdominal tenderness. A total score of 4 and above (with at least 3 score points from the first six items) was considered clinically significant for the classification of a subject within the GI group.

Moreover, all preschoolers were divided into regressive or non-regressive (early-onset -EO-ASD-) autism based on the presence/absence of a history of loss of competences such as language or social competences [50]; children belonging to regressive group were further divided in those with regression plus a previous developmental delay (Reg + DD) and those without a previous developmental delay (Reg − DD). According to Kern et al. [51], "regression *plus* developmental delay" was defined as a significant lag in the appearance of normal developmental milestones with a later loss of previously acquired skills.

This study was carried out according to the standards for good ethical practice and with the guidelines of the Declaration of Helsinki. The study protocol was approved by the Pediatric Ethics Committee of the Tuscany Region (Approval Number: 126/2014). Written informed consent from a parent/guardian of each participant was obtained.

### 2.2. Blood Sample Collection

A fasting blood sample (3 mL for each child) was collected in Ethylenediamine tetraacetic acid (EDTA) tube to perform the cytokines quantitative analysis. We didn't use pain patch before the sampling. Each tube was centrifuged for 10 min at 3500 rpm and all the plasma samples were stored at −80 °C until required the bio-humoral investigations

### 2.3. Cytokine Analysis

The cytokines were measured directly in the plasma through specific immunometric tests (MILLIPLEX MAP, human-magnetic bead panel, Millipore Corporation, Billerica, MA, USA) using an integrated multi-analyte detection platform (high-throughput technology Magpix system, Luminex xMAP technology, Luminex, Austin, TX, USA)

Each sample was analyzed in duplicate. In each one, a sample was analyzed as a quality control. Inter-assay variability was evaluated using two samples at different concentrations and was <10%.

### 2.4. Statistical Analysis

Descriptive statistics were computed for selected demographic variables across diagnostic groups. Contingency tables were used to perform the frequency analysis. Since the molecule's values were not normally distributed, we used log-transformed values with parametric statistic tests and non-parametric tests to compare GI vs. No-GI subjects (Mann-Whitney test) and to compare EO ASD vs. Reg-DD vs. Reg + DD (Kruskall-Wallis test) for all the selected molecules.

Correlation and regression analysis were computed to study the relationship between the molecules and the identified clinical parameters. Findings with $p$ value <0.05 were considered significant. StatView software (version 5.0.1; SAS Institute, Abacus Concept Inc., Berkeley, CA, USA) was used for data analyses. To discriminate different subgroups of ASD children based on biomarker levels, we performed Principal Component Analysis (PCA) using as correlated variables: sex, BMI, age, and cytokine levels (TNFa, IL6, CCL2, leptin, resistin and PAI 1). After log transformation and auto scaling (e.g., mean-centered and divided by standard deviation of each variable) PCA was performed using MetaboAnalystR 1.0.3 (Xia Lab, McGill University, Montreal, Canada). We checked quality control of samples using PCA that allowed us to label the 85 samples as outlier so it was excluded from downstream analysis.

## 3. Results

Thirty children (35%) were in the GI group and 55 (65%) in the No-GI group. Among the 30 GI subjects, 20 children (67%) were in the non-verbal group, whereas among the 55 No-GI, 26 children (47%) were in the non-verbal group. No statistically significant differences were found in the prevalence of GI subjects between verbal and non-verbal groups ($p = 0.086$). As concerns sex distribution, no differences were found in the prevalence of females in GI versus No-GI groups neither verbal versus non-verbal groups ($p = 0.560$ and $p = 0.804$, respectively).

As concerns clinical variables, there were no significant differences between the GI and the No-GI groups, with the exception of the Global Score of the RBS-R (60.24 ± 20.77 vs. 38.12 ± 27.06; $p = 0.0016$), the Internalizing and Total problem scores of the CBCL (all significantly higher in the GI group than in the No-GI group: 67.48 ± 7.80 vs. 62.06 ± 9.04, $p = 0.0065$ and 65.35 ± 10.02 vs. 60.62 ± 10.30, $p = 0.0469$, respectively), and of the Communication and Daily Living adaptive scores of the VABS (significantly higher in the No-GI group than in the GI group: 45.47 ± 15.22 vs. 54.46 ± 18.80 $p = 0.0274$ and 61.13 ± 14.29 vs. 69.07 ± 17.51 $p = 0.0365$, respectively).

As concerns proinflammatory cytokines levels, the single and the mean values in the total sample and in each subgroup are reported in Table 2. We did not find significant differences in the levels of plasmatic cytokines between GI and No-GI group except for resistin levels ($p = 0.032$). No difference in plasma biomarker levels was found between non-verbal and verbal groups.

Regarding the onset of autism, the mean values of cytokines were not statistically significant different between EO-ASD and regressive subgroups. Nevertheless, comparing cytokines levels in the EO-ASD subgroup with the two types of regressive preschoolers (with and without DD), resistin and PAI-1 levels were statistically significant higher in the Reg + DD group than in the other two groups, the EO-ASD and the Reg-DD ones ($p < 0.01$ for all).

Finally, after the correlation analysis between each molecule and all the clinical parameters, CCL2 levels negatively correlated with CBCL1.5-5 Internalizing and Total problems ($p = 0.0003$, $R = 0.383$ and $p = 0.013$, $R = -0.272$, respectively) and with RBS-R total scores ($p = 0.05$, $R = 0.21$), and positively correlated with VABS-II Motor Skills ($p = 0.019$, $R = 0.25$). TNF-α and PAI-1 levels negatively correlated with age ($p = 0.0005$, $R = -0.37$ and $p = 0.024$, $R = -0.25$, respectively); Leptin levels positively correlated with Body Mass Index ($p = 0.002$, $R = 0.34$) and negatively correlated with CBCL1.5-5 Internalizing problems ($p = 0.0086$, $R = -0.29$).

PCA analysis showed that the variability within the components explains the subdivision in clusters (No-GI vs. GI and EO-ASD vs. Reg − DD vs. Reg + DD) with a low percentage (PC1 = 21.3% and PC2 = 19.0%), indicating that the two and three groups respectively are not partially separated but overlapped (Figure 1).

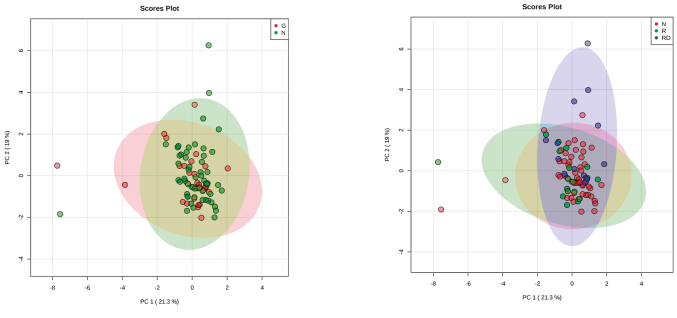


**Figure 1.** In the left plot, the Principal Component Analysis in gastrointestinal (red) and non-gastrointestinal subjects (green) is presented; in the right plot the PCA based on the ASD onset is presented: subjects with early-onset in red, regression without a previous developmental delay in green, regression plus a previous developmental delay in blue.

**Table 2.** Comparisons between the cytokine levels in GI vs. No-GI groups, in EO ASD (a) vs. Reg-DD (b) vs. Reg+DD (c) subgroups and No-Verbal vs. Verbal groups. The mean levels of each cytokine in the total sample are also reported.

| | | | | | a | b | c | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Total Sample** | **No-GI 55 Subjects** | **GI 30 Subjects** | ***p* (No-GI vs. GI)** | **EO ASD** | **Reg – DD** | **Reg + DD** | **ANOVA *p* Value** | **NO VERBAL 46 Subjects** | **VERBAL 39 Subjects** | ***p* (No-V vs. V)** |
| **N (%)** | 85 (100) | 55 (64.7%) | 30 (35.3%) | | 57 (67.0) | 14 (16.5) | 14 (16.5) | | | | |
| **TNF-α, m (SD) pg/mL** range 0.74–16.09 | 6.12 (2.40) | 5.84 (2.01) | 6.63 (2.95) | ns | 6.09 (3.16) | 6.76 (3.16) | 5.56 (2.56) | ns | 6.52 (2.57) | 5.63 (2.50) | ns |
| **IL-6, m (SD) pg/mL** range 0.80–104.00 | 5.99 (16.17) | 4.70 (13.83) | 8.34 (19.80) | ns | 5.74 (14.47) | 10.82 (27.24) | 2.18 (0.90) | ns | 4.67 (6.96) | 7.54 (22.69) | ns |
| **CCL2, m (SD) pg/mL** range 26.36–451.00 | 127.22 (58.81) | 131.61 (66.86) | 119.16 (39.90) | ns | 126.85 (56.03) | 135.10 (53.15) | 120.84 (76.74) | ns | 125.38 (53.73) | 129.39 (64.95) | ns |
| **Leptin, m (SD) pg/mL** range 0.03–4.83 | 1.14 (0.89) | 1.19 (0.96) | 1.06 (0.76) | ns | 1.26 (1.01) | 0.96 (0.50) | 0.88 (0.55) | ns | 1.01 (0.80) | 1.30 (0.97) | ns |
| **Resistin, m (SD) ng/mL** range 8.1–96.8 | 22.89 (13.63) | 24.50 (14.37) | 19.82 (11.74) | 0.032 | 20.97 (10.45) | 18.30 (9.68) | 35.14 (20.75) | 0.0003 (c > a) 0.0007 (c > b) | 23.65 (15.78) | 21.96 (10.60) | ns |
| **PAI-1, m (SD) ng/mL** range 5.5–91.2 | 26.04 (18.96) | 27.52 (20.27) | 23.24 (16.13) | ns | 23.28 (12.74) | 22.46 (14.04) | 40.67 (33.68) | 0.0018 (c > a) 0.0090 (c > b) | 28.66 (22.86) | 22.88 (12.32) | ns |

ns: not significant; Abbreviations (in alphabetic order): ASD: Autism Spectrum Disorder; CCL2: Macrophage Chemoattractant Protein-1; EO ASD: early onset of ASD without a history of loss of competences; GI: gastrointestinal; IL-6: interleukin-6; PAI 1: Plasminogen Activator Inhibitor-1; Reg – DD: regression without a previous developmental delay; Reg + DD: regression with a previous developmental delay; SD: standard deviation; TNF-α: Tumor Necrosis Factor-alpha.

## 4. Discussion

Our study fits within the complexity and the heterogeneity of studies that examine inflammation and immunity dysfunctions in ASD subjects, moving the field forward into the investigation of biological biomarkers to discriminate possible endophenotypes. The narrow age range considered, the detailed clinical characterization with specific and gold-standard tools for ASD evaluation, and an enough large sample represent the strengths of the study.

First, we found that the single and the mean values of our cytokines were lower than those expected in subjects with systemic inflammation [52–54]. These findings are in agreement with a part of the literature on this topic in which there is an absence of any atypical profile in the expression of relevant plasma cytokines both within ASD subjects and in comparison with TD children [55]. Regarding plasmatic cytokines, it should be highlighted that in literature the reference values and in particular those relating to the pediatric age, to date, are not definitively characterized. Despite our attempt to define specific subgroups based on cytokines levels and anthropometric measures using PCA, in our sample different endophenotypes were not identified. These results exclude the possibility that bringing all cases together in a single ASD group could have hidden significant results in one specific subgroup of preschoolers, as previously hypothesized [56,57]. Consequently, our findings do not support the use of anti-inflammatory therapies in ASD children, not even in a specific subgroup of ASD subjects as previously suggested [58].

Second, we did not observe significant differences in the levels of circulating cytokines between GI and No-GI ASD children, except for resistin. Notably, there is too scant relevant research on this topic in ASD subjects [29,39] to draw valid and accurate conclusions. Thus, the role of adipokines needs further studies, in particular, in correlation with GI symptomatology in ASD considering also the influence of fat mass in plasmatic levels of adipokines. These findings suggest that the frequently reported GI symptoms in ASD children seem to be independent from an inflammatory condition, confirming a not yet clarified meaning of these symptoms [59]. Previously, only a modest relationship between GI symptoms and TNF-α levels was detected [17,28], in one case [28] in significantly older subjects (school-aged children and adolescents) than ours. Specifically, when Ferguson et al. [28] considered only inferior GI symptoms (as we did) they did not identify any statistically significant correlations, in line with the findings that TNF-α levels are independent from the presence of GI symptoms [22,60]. Some authors [61–64] have measured the presence of cytokine-producing cells directly in the bowel of subjects with ASD, and found a local high level of these cells in patients with GI symptoms, supporting a local role of the inflammatory cytokines in altering intestinal epithelial barrier and thus in contributing to GI symptoms. Besides, we confirm our previous findings showing that ASD subjects with GI problems have worse clinical functioning than ASD subjects without GI problems, independently from the severity of autistic symptoms [65].

We did not find any significant correlations between the basal levels of TNF-α and IL-6 and the autistic features of the total sample, similarly to some investigations [56,66] and in contrast to others [17,28,67,68]. Moreover, we found a positive, though weak, correlation between CCL2 and better functioning of children, evaluated with the CBCL1.5-5, RBS-R and VABS-II, in contrast with studies reporting a significant correlation between higher CCL2 plasmatic levels and more severe impairment of the autistic condition [21,57,69]. Further studies are necessary to disentangle the controversial findings on the possible role of some cytokines as sensible markers of the impairment in ASD children.

Third, we found that the group with regression plus developmental delay prior to the onset of ASD (16.5% of the sample) was significantly different from the rest of the sample as far as the higher plasmatic levels of resistin and PAI-1. We could suggest that Reg + DD children represent a specific subgroup with a definite biological profile and a specific clinical feature. However, using the PCA method, we did not identify the Reg + DD group as a particular cluster of patients, making the individuation of a specific endophenotype unlikely in this sample. Future studies are needed to retest the robustness of these findings before we can consider them as reliable.

In addition, we did not identify any significant correlation between the levels of cytokines and the presence or absence of a regression of skills prior to the onset of autism. This result is in accordance with the majority of similar investigations, but in contrast with others where an association, although weak, between regressive autism and TNF-α [70], or lower plasma leptin levels [34] was found. Previous studies detected higher basal plasmatic levels of IL-1β [17,69], IL-5 [69], IL-17 [69] and higher levels of neural cell adhesion molecule (NCAM) [55]—a molecule playing a role in cell–cell adhesion, neurite outgrowth, synaptic plasticity, learning and memory—in subjects with a regression of skills prior to the onset of autism. More broadly, ASD subjects with regression have been repeatedly identified as different in pathophysiological findings from ASD subjects without regression both in terms of neuroanatomy [71], and EEG patterns [72]. However, there is an urgent need to study the clinical regression in ASD, since a clear understanding of the definition, prevalence, etiopathogenesis, age of onset, and outcome profiles of this complex phenomenon is far from being concluded [73,74].

*Limitations*

We must consider this study as a pilot investigation with several limitations. Compared to other authors who have measured a series of pro-inflammatory cytokines in ASD subjects [22], we focused our analysis on six cytokines, so limiting the possible range of our results. The changes in the expression of cytokines due to subjects' age [75] have already been described, and we cannot exclude that our results on inflammatory markers could be age-specific; in addition, we have to consider that sex, sleep-wake cycle and the percentage of fat mass, which could increase that variability [76,77] representing possible interfering factors, have not been assessed in this study. Moreover, the low number of females within our sample of preschoolers with ASD did not allow us to accurately investigate possible sex differences in pro-inflammatory cytokine profiles.

## 5. Conclusions

Despite the above-mentioned limitations and the existing controversies within the studies about the role of cytokines in ASD and the extreme variability of their findings, our study finds no evidence of the presence of inflammatory condition in ASD subjects, except for resistin. Our findings do not support the use of anti-inflammatory therapies in ASD children, and paves the way for the search of alternative hypotheses for the etiology of GI symptoms in subjects with ASD. Despite our findings showed a specific plasmatic cytokine profile in ASD children with a history of a regressive way of onset within a previous developmental delay, the specific endophenotype for these subjects has not been identified.

*Ethics Approval and Consent to Participate*

The study protocol was approved by the Pediatric Ethics Committee of Tuscany Region (Protocol Number: 126/2014), with written informed consent obtained from a parent/guardian of each participant. The study was conducted following the 1964 Declaration of Helsinki and its later amendments, and the International Conference on Harmonisation Guidelines for Good Clinical Practice.

**Availability of Data and Material:** The datasets generated and/or analyzed during the current study are not publicly available due to the privacy policy (containing information that could compromise research participant privacy/consent) but are available from the corresponding author on reasonable request and with permission of parents of the involved children.

**Author Contributions:** Conceptualization and Methodology, F.M., L.G., M.A.M. and E.S.; Laboratory Analysis, L.G. and M.G.; Investigation, M.P., L.G. and E.S.; Formal Analysis, L.G. and C.N.; Resources, F.M., L.G., M.A.M. and E.S.; Data Curation, M.P. and C.N.; Writing – Original Draft Preparation, M.P.; Writing – Review & Editing, L.G., D.G.P., C.N., S.C., R.T., M.A.M., A.G., F.M. and E.S.; Supervision, D.G.P., M.A.M., A.G. and F.M; Funding Acquisition, F.M. All authors read and approved the final manuscript.

**Funding:** This trial is funded by the Italian Ministry of Health and by Tuscany Region with the grant 'GR-2011-02348280'. This work was also partially supported by grants from the IRCCS Stella Maris Foundation (Ricerca Corrente, and the "5 × 1000" voluntary contributions, Italian Ministry of Health to F.M., R.T., S.C. and E.S.). We are also grateful to Università di Pisa for supporting Dr. Prosperi with a research Grant (D.R. n. 33134 29/05/2018).

**Acknowledgments:** We gratefully acknowledge the families participating in our research.

**Conflicts of Interest:** The authors declare no conflict of interest.

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# Paternal—but Not Maternal—Autistic Traits Predict Frontal EEG Alpha Asymmetry in Infants with Later Symptoms of Autism

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Received: 21 October 2019; Accepted: 24 November 2019; Published: 26 November 2019

**Abstract:** Previous research found that the parental autism phenotype is associated with child autism spectrum disorder (ASD), even if the pathway between autistic traits in parents and child ASD is still largely unknown. Several studies investigated frontal asymmetry in alpha oscillation (FAA) as an early marker for ASD. However, no study has examined the mediational effect of FAA between parental autistic traits and child ASD symptoms in the general population. We carried out a prospective study of 103 typically developing infants and measured FAA as a mediator between both maternal and paternal autistic traits and child ASD traits. We recorded infant baseline electroencephalogram (EEG) at 6 months of age. Child ASD symptoms were measured at age 24 months by the Child Behavior Checklist 1½–5 Pervasive Developmental Problems Scale, and parental autistic traits were scored by the Autism spectrum Quotient questionnaire. The mediation model showed that paternal vs. maternal autistic traits are associated with greater left FAA which, in turn, is associated with more child ASD traits with a significant indirect effect only in female infants vs. male infants. Our findings show a potential cascade of effects whereby paternal autistic traits drive EEG markers contributing to ASD risk.

**Keywords:** autism spectrum disorder; infants; frontal EEG alpha asymmetry; early detection

## 1. Introduction

Autism spectrum disorder (ASD) is a complex and heterogeneous condition characterized by social communication deficits and repetitive patterns of behavior [1]. Twin studies show that ASD is a heritable condition, with heritability estimates ranging between 64% and 91% [2]. Genetic susceptibility appears to be expressed in relatives of individuals with ASD through an independent segregation of a broader range of subclinical features (autistic traits) in social communication and atypical patterns that are referred to as representing the broader autism phenotype (BAP). Several studies demonstrated that autistic traits are distributed normally in the general population and are heritable [3]. In particular, parental autistic traits have been found associated with child ASD symptoms in both ASD samples and the general population, suggesting that broader autistic traits are important to identify both clinical and subclinical conditions [3]. However, even if BAP and ASD seem to exist on a continuum, it is still unknown whether and how maternal and paternal autistic traits are differentially associated with

child social communication development. Several studies examined sex-specific associations between parental autistic traits and child ASD symptoms [4–6]. For example, Schwichtenberg et al. [5] found that paternal autistic traits predicted child ASD severity, while this relationship was not found for mothers. Klusek et al. [7] showed that paternal autistic traits (rigid and untactful traits) were associated with more social deficits in their ASD children. Conversely, a significant association emerged between child performance on a facial identity recognition task and maternal autistic traits, with no relationship between fathers and their children's scores [6]. Overall, the idea that paternal characteristics are more strongly associated with child ASD phenotype than maternal characteristics is more consistent with the literature, but it has not been well replicated.

Parental autistic traits are also relevant for the evaluation of endophenotypes [8]. A study using an eye-tracker system in 8-month-old infants found that paternal autistic traits were associated with infants' attentional functioning, suggesting that early impairments in low-level attentional systems may affect high-level social impairment [9].

Although it has been clearly established that child ASD traits can be influenced by parental autistic traits, the underlying mechanisms and the pathway between parental autistic traits and ASD symptoms in children are still largely unknown. Previous electroencephalogram (EEG) studies showed that anomalous oscillatory organization in multiple frequency bands was strongly associated with ASD, with many of these studies emphasizing the crucial role of individual changes in frontal EEG alpha power [10]. Alpha-band oscillations are associated with precise timing of sensory and cognitive inhibition [11]. Interestingly, significant differences in baseline alpha power were identified in infants at high risk for ASD (siblings of children with ASD) compared to typically developing infants, whereby high-risk infants at age 6 months showed lower alpha power as compared to controls [11].

In addition to spectral power differences, changes have been reported in hemispheric asymmetry of the frontal alpha band. Frontal alpha asymmetry (FAA) refers to the difference in EEG power between the frontal right hemisphere and the frontal left hemisphere [12]. Differences in alpha activity between the left and right hemispheres have been used to measure neural activity as a metrics for frontal lobe organization. In particular, because alpha power is inversely related to cortical activation (meaning that decreased alpha power reflects greater brain activity), right FAA indicates higher cortical activation in the right hemisphere and left FAA indicate higher cortical activation in the left hemisphere. In other words, positive values are associated to left FAA (left hemisphere activation) and negative values are associated to right FAA (right hemisphere activation).

FAA has been found associated with ASD [13,14]. Sutton and colleagues [14] examined the relationship between FAA, social impairment, and social anxiety in a sample of high-functioning ASD children compared to controls. The groups significantly differed on FAA, social impairment, and anxiety symptoms. ASD children with right FAA displayed more social deficits and ASD symptoms, whereas greater left FAA was associated with less social deficits and more anxiety symptoms.

Interestingly, research with typically developing infants has provided evidence that FAA changes during early years of life. Typically developing infants gradually shift from right FAA at age 6 months to left FAA at age 18 months [15–18]. Interestingly, recent evidence indicates that 6-month-old infants at high risk for ASD show an opposite developmental trajectory in FAA, shifting from left FAA at age 6 months to right FAA by age 18 months [17]. Overall, these data demonstrated a different hemispheric organization in infants at high risk for ASD, whereby frontal asymmetry may represent one of the earliest potential endophenotypes for ASD. Despite the fact that FAA has been found associated with ASD [16,17] and parental autistic traits are associated with child ASD [8], no study has explored the role of autistic traits in mothers and fathers on FAA and later child ASD symptoms concurrently.

In addition, recent growing evidence suggests that the pattern of frontal EEG asymmetry might be associated with psychological processes in female infants to a greater extent than male infants. Indeed, there is strong evidence that the link between FAA and psychosocial difficulties may be moderated by sex, with stronger associations in female infants than male infants [19,20]. Even if there are no studies

in the ASD field, it may be interesting to explore the relationship between FAA and ASD symptoms in female infants and male infants separately.

Based on this, we conducted a prospective longitudinal study on 103 typically developing infants (general population) and investigated FAA at age six months as a possible mediator of the impact of parental autistic traits on child ASD symptoms. The study aimed to determine 1) whether parental autistic traits are associated with FAA at age six months and child ASD symptoms at age 24 months 2) whether six-month FAA may reflect a potential neural mechanism predicting child ASD symptoms at age 24 months, 3) whether 6-month FAA is a mediator of the contribution of parental autistic traits to ASD-related symptoms, and 4) whether child sex moderates the associations between parental autistic traits, FAA, and ASD symptoms. Our assumption was that FAA significantly predicts child ASD traits and that FAA would serve as a potential neural mediator between parental autistic traits and child ASD-outcome. Based on previous research indicating the influence of sex on the FAA link to developmental psychopathology, we hypothesized that the tested mediation model is moderated by child sex, with a stronger effect in female infants than male infants.

## 2. Materials and Methods

### *2.1. Sample*

At 6 months of age, 103 typically developing infants took part in the study (female-to-male ratio = 0.51). The sample was recruited from two hospitals in Northern Italy [21,22]. Inclusion criteria were (a) gestational age ≥36, (b) birth weight ≥2500 g, (c) Bayley Cognitive Score at 6 months ≥7 [23], and (d) no certified intellectual disabilities among first-degree relatives. Families with a diagnosis of ASD among first-degree relatives were also excluded, since we decided to focus on a broad autism phenotype in the general population. Descriptive statistics of demographics and clinical characteristics are shown in Table 1. Parents of all children had given their informed consent for inclusion before participation in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of IRCCS Medea (Ricerca Corrente "2016, 2017, 2018, 2019", Ricerca Finalizzata "NET-2013-02355263-2" and "5 per mille" funds for biomedical research).

**Table 1.** Descriptive statistics of demographics and clinical characteristics.

| | **Total Sample (*n* = 103)** | **Males (*n* = 50)** | **Females (*n* = 53)** |
|---|---|---|---|
| | **Mean (±SD)** | **Mean (±SD)** | **Mean (±SD)** |
| Birthweight (grams) | 3252.20 (±468.08) | 3325.33 (±481.35) | 3179.07 (±453.03) |
| Gestational age (weeks) | 39.08 (±1.47) | 39.67 (±1.27) | 38.49 (±1.66) |
| Maternal educational level [a] | 58.04 (±17.05) | 56.50 (±19.39) | 59.52 (±14.49) |
| Paternal educational level [a] | 49.31 (±17.68) | 50.41 (±17.67) | 48.27 (±17.79) |
| Socioeconomic status [b] | 61.47 (±15.51) | 61.10 (±15.63) | 61.83 (±15.53) |
| Bayley cognitive subscale at 6 months [c] | 12.07 (±1.81) | 11.82 (±1.96) | 12.30 (±1.65) |
| Paternal AQ (raw scores) | 17.81 (±6.22) | 19.20 (±6.49) | 16.44 (±5.68) |
| Maternal AQ (raw scores) | 14.49 (±5.77) | 15.28 (±5.95) | 13.73 (±5.53) |
| Paternal AQ [d] | −0.26 (±1.37) | −0.53 (±1.40) | 0.003 (±1.22) |
| Maternal AQ [d] | 0.03 (±1.09) | −0.03 (±1.16) | 0.10 (±1.02) |
| CBCL 1½-5 Pervasive Developmental Problems [e] | 53.42 (±5.94) | 52.84 (±4.88) | 53.98 (±6.81) |

[a] The educational level of mothers and fathers was scored on a 9-point ordinal scale created ad-hoc and based on the Italian school system. [b] Socioeconomic status was scored according to Hollingshead 9-point scale, whereby a score ranging from 10 to 90 was assigned to each parental job and the higher of two scores was considered when both parents were employed [24]. [c] Age-standardized (mean = 10; SD = 3) score on the Bayley cognitive subscale [23]. [d] Age-standardized *z*-scores (mean = 0; SD = 1) for the total Autism Spectrum Quotient (AQ) score [25]. [e] Age-standardized T-scores (mean = 50; SD = 10) for the Child Behavior Check List 1½–5 (CBCL 1½–5) [26].

### *2.2. Frontal EEG Alpha Asymmetry*

#### 2.2.1. EEG Data Acquisition

Four minutes of baseline EEG at age 6 months (M = 6.46 months; SD = 0.49) were used to compute alpha asymmetry scores. Baseline EEGs were recorded after an experimental session (i.e., a passive oddball paradigm intended to test auditory processing skills; see [27]. During EEG recording, infants were looking at an experimenter blowing soap bubbles.

#### 2.2.2. EEG Data Processing and Analysis

EEGs were recorded from 60 scalp electrodes using HydroCel Geodesic sensor nets (Electrical Geodesics, Inc., Eugene, Oregon, USA). The vertex electrode was the online reference. EEGs were recorded with a sampling rate of 250 Hz and an online band-pass filter (0.1–100 Hz). After recording, EEGs were exported and further processed using lab-internal MATLAB (Mathworks, Natick, MA, USA) routines and the EEGLAB toolbox [28]. Data were band-pass filtered at 1–47 Hz. Bad channels were identified by means of the EEGLAB "TrimOutlier" plugin (identification criteria: 5 < all channels SD < 100) and interpolated with a spherical spline (a maximum of 12 out of 60 channels were interpolated, M = 3.3, SD = 2.7). Data were then re-referenced offline to an average reference and segmented in one-second non-overlapping epochs. Bad EEG epochs were identified and rejected using two EEGLAB functions: (1) "find abnormal values", marking for rejection epochs in which EEG values exceeded ±150 μV) and (2) "find abnormal trends", marking for rejection epochs corrupted by linear drift (setting parameters: R = 0.3, max slope = 150 μV). Additional manual artifact inspection was computed. A minimum of 60 artifact-free segments (M = 119.8; SD = 39.9) was used for subsequent power analysis. Power spectral density (PSD) was estimated by Welch's method [26,29] with non-overlapping 0.5 s windows. PSD values were calculated for each channel in each epoch and then averaged across segments. Following previous literature [17,30], the mean power in the infant alpha frequency band (6–9 Hz) was computed. We selected two clusters of electrodes (based on and adapted from [17], frontal left hemisphere: 9, 11, 12, 13, 14 and frontal right hemisphere: 2, 3, 57, 59, 60 (see Supplementary Materials) and power values were averaged across electrodes within each cluster. In full-spectrum data, we focused on frontal alpha asymmetry (FAA) that has been well characterized in infants. Frontal asymmetry scores were calculated from log-transformed PSD values in selected clusters as follows: (right − left)/(right + left). This formula has been used in most studies to summarize the relative activity at homologous right and left sites [31]. Use of this formula to calculate FAA offers the advantage of minimizing bias due to individual differences in skull thickness that might influence the power spectrum amplitude. In addition, it approaches a normal distribution and shows good stability and reliability [32]. Positive values indicate left FAA and negative values indicate right FAA (M = 0.03; SD = 0.13).

#### 2.2.3. Autistic Traits in Parents: The Autism Spectrum Quotient

The Autism Spectrum Quotient (AQ) is a self-administered questionnaire to quantify autistic traits in the general population [25], with the Italian version by [33]. The AQ questionnaire offers several advantages, including subscales tapping both social and non-social aspects of behavior and cognition and a brief, self-administered, and forced-choice format [34]. Subjects are instructed to respond to each of the 50 items using a 4-point Likert scale as follows: "definitely agree", "slightly agree", "slightly disagree", and "definitely disagree". The maximum score on the AQ is 50 points, with higher scores meaning higher presence of autistic traits. Two cut-offs were previously described/reported [25]: clinical threshold (raw scores ≥ 32) and screening cut-off (raw scores ≥ 26). Reflecting the non-clinical nature of our sample, only 3 parents (all fathers) reached the clinical threshold and only 13 parents (9 fathers and 4 mothers) reached the screening cut-off. The AQ questionnaire was completed by both parents upon their children's inclusion in the study. Total AQ scores were transformed into *z*-scores (see Table 1) and were used in further analysis.

#### 2.2.4. Autistic Traits in Infants: The CBCL 1½–5 Pervasive Developmental Problems Scale

The Child Behavior Check List 1½–5 (CBCL 1½–5) consists of 99 items designed to rate emotional and behavioral problems in toddlers. Items are scored by parents on a 3–point Likert scale (0 = not true; 1 = sometimes true; 2 = very true) and they refer to a time span of 6 months before the questionnaire completion. In our sample, 73 questionnaires were filled by mothers (80%), 5 by fathers (5%), and 14 by both parents (15%). This measure with strong psychometric properties across cultures has been translated into, and validated in Italian [35,36]. For the purpose of this study, the Pervasive Developmental Problems scale (PDP) was used as child ASD symptoms and PDP T-scores (mean = 50; SD = 10) were used in the analysis. Reflecting the non-clinical nature of our sample, only 7 children (4 female infants and 3 male infants) reached the clinical threshold (T ≥ 65).

### *2.3. Statistical Analysis*

Descriptive statistics and Pearson's bivariate correlations to examine the associations among study variables were run using SPSS, Version 25.0 (IBM Corp., Armonk, NY, USA). The association between FAA and ASD-related traits was assessed by linear regression analysis: the CBCL 1½–5 PDP score was entered as the dependent variable and FAA was set as the predictor.

To investigate the contribution of paternal and maternal AQ to ASD-related traits in children and the potential role of FAA as a mediator, we used Structural Equation Modeling (SEM) [36], as implemented in the MPLUS software (Los Angeles, CA) [37]. SEM simultaneously models all paths, providing a more accurate estimation of mediation effects [38,39] than more traditional tests based on sequential regressions.

The mediation model tested the hypothesis that ASD-related traits in children would be explained by a sequence of potentially associated effects involving parental autistic traits and FAA. Specifically, the following model was proposed: maternal and paternal AQ → FAA → child ASD traits. We then assessed the mediation model which best described the associations between the measured variables [39]. Finally, moderation by child sex was examined to assess whether relations between study variables differed by sex (male vs. female).

The bias-corrected 5000 bootstrap technique was used to test mediation effects [36]. Confidence intervals (95% CI) that do not contain zero indicate significant indirect effects [40–43]. Several fit indices are used to assess the best fitting model: a) Chi-Square assessing the difference in magnitude between the model estimated variance/covariance matrices, b) the RMSEA (root mean square error of approximation) considering the complexity of the model [42], c) the SRMR (standardized root mean square residual) indicating the average residual value from the model fit covariance matrix to the sample covariance matrix, and d) the CFI (comparative fit index) indicating the improvement in overall model fit by comparing the hypothesized model with a more restricted one, which specifies no relations among variables. RMSEA ≤ 0.05, SRMR ≤ 0.08, and CFI ≥ 0.95 indicate a good model fit [42–45]. To allow for the use of all available data with inclusion of subjects with missing data, we considered the full information maximum likelihood estimation. Significant effects were set to $p$-values ≤ 0.05.

## 3. Results

We first examined the correlations between maternal and paternal AQ (z-scores), CBCL 1½–5 Pervasive Developmental Problems (T-scores), and FAA. We found a significant correlation between FAA and CBCL 1½–5 PDP scores ($r = 0.42$, $p < 0.001$), with greater left FAA being associated with more child ASD-related symptoms. Correlations between paternal AQ scores and FAA ($r = -0.29$, $p = 0.003$) and paternal AQ scores and CBCL 1½–5 PDP scores were low-to-moderate ($r = -0.39$, $p < 0.001$). Higher paternal autistic traits (negative z-scores for AQ values) were associated with greater left FAA at age 6 months and more child ASD-related symptoms at age 24 months. No significant correlation emerged between maternal AQ scores and FAA ($r = -0.11$, $p = 0.244$) and a low—although not significant—correlation was found between maternal and paternal AQ scores ($r = 0.14$, $p = 0.157$).

Finally, no significant correlation was found between maternal AQ scores and CBCL 1½–5 PDP scores ($r = -0.09$, $p = 0.394$).

### *3.1. Testing a Mediation Model: FAA as a Mediator between Parental AQ Scores and Child ASD-Related Traits*

After carrying out descriptive and correlational statistics, we used SEM to test the mediation model shown in Figure 1, which assumes that maternal and paternal AQ scores contribute to FAA. FAA, in turn, affects child ASD-related traits. The model provided a good fit to the data ($\chi^2$ (5) = 37.31, $p < 0.001$; RMSEA = 0.000, CI (90%) = 0.000–0.000; CFI = 1.00; SRMR = 0.000) and accounted for 26.3% of the variance in CBCL 1½-5 PDP scores. Figure 1 shows standardized coefficient estimates. The mediation model yielded several significant direct effects. There was a significant path coefficient from paternal AQ to CBCL 1½–5 PDP scores (β = −0.30, $p = 0.004$). Children with higher paternal autistic traits showed higher PDP scores at age 24 months. No significant association from maternal AQ to CBCL 1½–5 PDP scores emerged (β = −0.03, $p = 0.719$). Significant effects were found from paternal AQ scores to FAA (β = −0.28, $p = 0.014$), and from FAA to CBCL 1½–5 PDP scores (β = 0.33, $p = 0.028$): Higher paternal autistic traits predict greater left FAA and greater left FAA predicts higher infant ASD-related traits. However, 5000 bootstrap estimates (95% CI) showed that the indirect effect from paternal AQ to CBCL 1½–5 PDP scores via FAA was not significant (β = −0.092; SE = 0.256; 95% CI (−0.193; 0.010), $p = 0.112$).

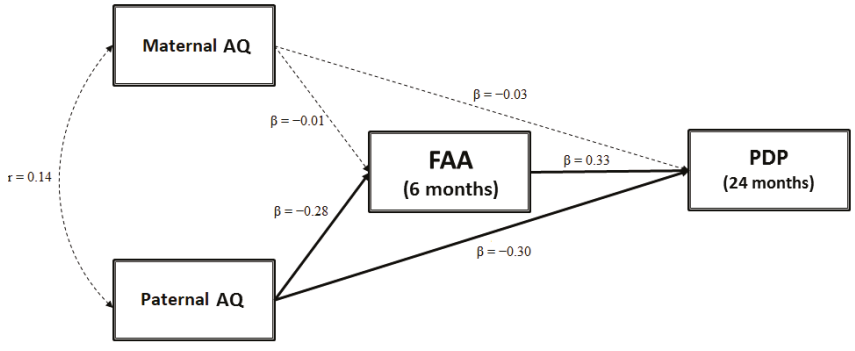


**Figure 1.** Frontal asymmetry in alpha oscillation (FAA) as a mediator between maternal and paternal autism spectrum quotient (AQ) scores and child autism spectrum disorder (ASD)-related traits. PDP—Pervasive Developmental Problems.

### *3.2. Testing a Moderated Mediation Model: FAA as a Mediator between Parental AQ and Child ASD-Related Traits Moderated by Sex*

Moderated mediation was also applied to examine whether child sex moderated the associations between maternal and parental AQ scores, FAA, and child ASD-related outcome via multigroup analyses in the SEM framework (see Figure 2 for group-specific parameter estimates).The model provided a good fit to the data ($\chi^2$ (10) = 44.74, $p < 0.001$; RMSEA = 0.000, CI (90%) = 0.000–0.000; CFI = 1.00; SRMR = 0.000) and accounted for 15.5% of the variance in the CBCL 1½–5 PDP scores in male infants and 40.5% of the variance in the CBCL 1½–5 PDP scores in female infants.

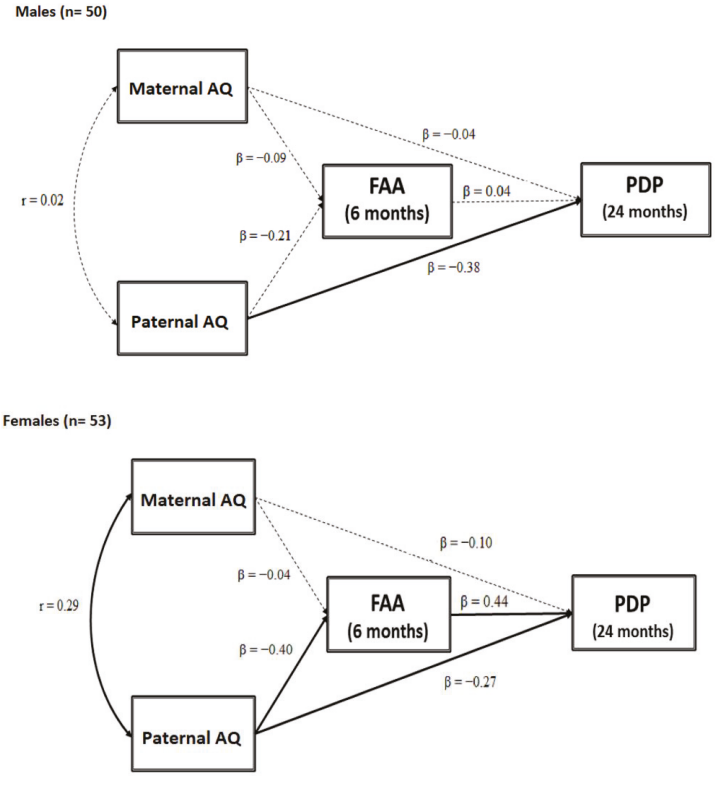


**Figure 2.** FAA as a mediator between maternal and paternal AQ and child ASD-related traits moderated by sex.

Standardized estimates of path coefficients in each group are depicted in Figure 2. In male infants, there was only a direct effect of paternal AQ to CBCL 1½–5 PDP scores (β = -0.38, $p = 0.027$), with higher paternal autistic traits predicting higher CBCL 1½–5 PDP scores. No other direct or indirect effect was found.

In female infants, significant associations were found from paternal AQ scores and FAA (β = −0.40, $p = 0.010$), from paternal AQ scores and CBCL 1½–5 PDP (β = −0.27, $p = 0.038$) and from FAA to CBCL 1½–5 PDP scores (β = 0.44, $p = 0.014$): Higher paternal autistic traits predicted greater left FAA which, in turn, predicted higher CBCL 1½–5 PDP scores. A significant correlation between maternal and paternal AQ scores ($r = 0.29$; $p = 0.041$) was also found.

Interestingly, 5000 bootstrap estimates (CI 95%) showed that the indirect effect from paternal AQ to CBCL 1½–5 PDP scores via FAA was significant for female infants (β = −0.176; SE = 0.579; 95% CI (−0.334; −0.016), $p = 0.041$) but not for male infants (β = −0.008; SE = 0.117; 95% CI (−0.251; 0.246), $p = 0.815$). In female infants, paternal AQ scores were associated with greater left FAA at age 6 months, which affected child ASD-related traits at age 24 months. In other words, FAA mediates the association between paternal AQ scores and child ASD-related symptoms, and this link was moderated by child sex in female infants but not in male infants (for graphical purposes only, see Figure 3).

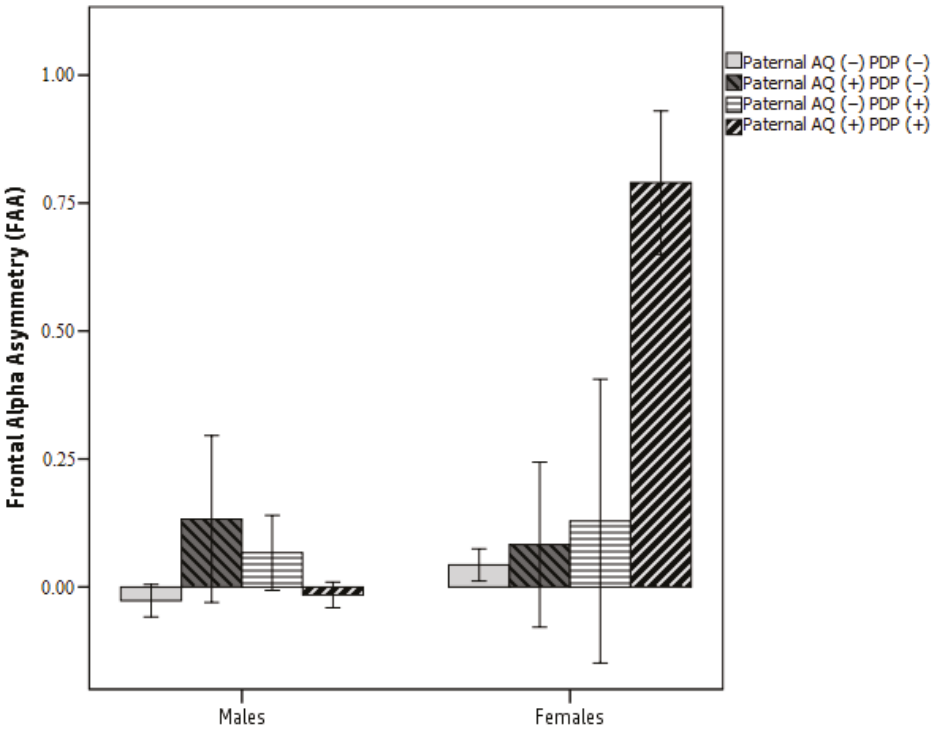


**Figure 3.** FAA differences between paternal AQ scores, CBCL 1½–5 PDP scores and child sex. Legend: Paternal AQ scores (−): low paternal autistic traits; Paternal AQ scores (+): high paternal autistic traits; PDP scores (−): low CBCL 1½–5 PDP scores; PDP scores (+): high CBCL 1½–5 PDP scores.

## 4. Discussion

This is the first general population study looking at frontal asymmetry in EEG alpha oscillation at age 6 months as a potential mediator in the developmental pathway from maternal and paternal autistic traits to child ASD-related traits at age 24 months.

### *4.1. Parental Autistic Traits and Child ASD Symptoms*

Not surprisingly, paternal autistic traits were associated with more child ASD-related symptoms, supporting the assumption that broader autistic traits in parents may be useful in identifying both clinical and subclinical conditions [3]. This finding is well replicated in previous studies. The association between higher autistic traits in parents and their children strongly supports an underlying genetic mechanism [46]: Shared genetic variability may be a plausible pathway for familial transmission of common factors between parental autistic traits and their child ASD-related traits. Consistent with the literature, we found that paternal characteristics are associated with child ASD phenotype rather than maternal characteristics [4–6], supporting greater patrilineal effects within families [7].

### *4.2. Parental Autistic Traits and Frontal Asymmetry in Alpha Oscillation*

Our findings also showed that higher paternal autistic traits were associated with greater left FAA at age 6 months. Typically developing infants shifted from greater right FAA (right frontal activation) at age 6 months to relative greater left FAA (left frontal activation) at age 18 months [15,16]. Interestingly, an opposite pattern was found in infants at high risk for ASD (siblings of children with ASD) from greater left FAA at age 6 months to greater right FAA at age 18 months [17,18,47]. In line with this piece

of evidence, we found that at-risk infants (having a parent with higher autistic traits) aged 6 months showed greater left FAA, suggesting an atypical organization and lateralization of oscillatory processes. We might speculate that hemispheric organization follows a different developmental shift in infants with higher paternal autistic traits. However, since EEG measures at age 18 months were not obtained, further studies are needed to confirm this hypothesis.

### *4.3. Frontal Asymmetry in Alpha Oscillation and Child ASD Symptoms*

Greater left FAA at age 6 months was associated with increased child ASD-related traits at age 24 months. Past research has linked FAA to different cognitive and behavioral processes, supporting the role of FAA as a biomarker for psychopathology [48]. FAA is a measure of the propensity to adopt an "approach or withdrawal" behavior [48], with greater left frontal activity associated with an increased tendency to approach, and greater right frontal activity associated with an increased tendency to withdraw. Taken together, frontal alpha asymmetry can be interpreted with respect to the amount of motivation towards (approach) or away from (withdraw) something or someone. Relating to ASD, greater right frontal asymmetry is associated with social impairment and earlier onset of ASD symptoms [13,14], whereas less social impairment has been observed in children with greater left frontal asymmetry.

However, the direction of the effect that we found in the present study (i.e., greater left FAA at age 6 months associated with earlier ASD-related symptoms) was different from what was expected based on previous literature. This discrepancy could be due to different population characteristics (infants vs. children/adolescents; typically developing infants vs. high-functioning ASD children). Since frontal asymmetry tends to change over the first two years of life, with a shift in lateralization from right to left FAA in typically developing infants and from left to right FAA in infants at risk for ASD [15], the different direction of the reported effects between the present and previous studies might be due to maturation effects. This needs to be confirmed in more overlapping study populations.

### *4.4. Frontal Asymmetry in Alpha Oscillation as a Mediator between Paternal Autistic Traits and Child ASD Symptoms*

Perhaps more importantly, we found that paternal, but not maternal, autistic traits are directly associated with FAA and FAA directly affects child ASD-related traits. This different association may reflect different biological mechanisms based on parent-of-origin effects, namely the genetic effects on the (endo)phenotype of an offspring that are dependent on the parental origin of the associated genetic variants. Several studies [49,50] found that parents may transmit genes or epigenetic dysregulation affecting ASD through sex-specific pathways and, in line with our own results, parent-of-origin effects were found in ASD, with a paternal over-transmission of risk alleles for ASD [49]. Even if further research is needed, our results may support the importance to explore the role of epigenetic modulators in the etiology of ASD. If parent-of-origin effects are proven, more homogeneous ASD-related phenotypes could be identified by grouping according to parental ASD traits.

Although in an exploratory manner, we found a significant indirect effect and provided the first evidence that FAA at age 6 months significantly mediates the contribution of paternal autistic traits to ASD-related traits in their children, while this is seen at age 24 months in female infants but not in male infants. It is well replicated that male infants are more frequently diagnosed with ASD than female infants, with a reported sex ratio of 4:1 [51]. Sex differences may reflect the distinctive sexual dimorphism of the brain, including hormonal and structural factors as well as genetic and epigenetic influences, which emerge during development. For example, effects of serotonin genotypes on EEG activity were found to vary as a function of sex. The 5-HTTLPR polymorphism was associated with modulation of the EEG activity at different EEG frequencies only in female infants and not in male infants [52], suggesting that baseline EEG frontal activity marks different neurobiological processes in female infants and male infants. Understanding the mechanisms underlying the sex difference in ASD is not only fundamental per se, but it might crucially contribute to unravelling the well-known

sex differences in prevalence, age of onset, and severity that we observe in many psychiatric diseases, including depression and anxiety disorder and ADHD, in which a role of FAA has been reported [20,53]. Even if replication studies are necessary, it is conceivable that FAA involved in cortical development—if combined with higher parental autistic traits—could potentiate different genetic vulnerabilities in male infants and female infants, specifically ASD-related problems.

This study presents some limitations. First, ASD traits were assessed solely by parental report. Although no evidence for report bias regarding parent–offspring autistic traits emerged in previous studies [54], in our study we cannot exclude that parental ASD traits might have an effect on parental perception of their child's behavior. Therefore, we suggest that future studies should focus on direct assessment of autism-related symptoms. Second, we measured EEG frontal alpha asymmetry only at 6 months of age. As reported by previous studies [15–17], FAA seems to be developmentally sensitive from age 6 to 24 months. Future longitudinal studies with larger samples of typically developing and at-risk infants are important to increase our confidence on (a) typical EEG asymmetry trajectories and how such EEG trajectories relate to different broader autism phenotype domains. A further point to be highlighted concerns the specificity of the relationship between FAA and ASD traits. Previous studies reported that oscillations in different frequency bands are related to the development of other cognitive skills (i.e., oscillations in the gamma frequency bands have been reported to be predictive of language skills) [55]. In our study, we tried to disentangle a possible connection between frontal alpha asymmetry and language by means of exploratory correlations with language measures at 24 months and found—as expected—no significant correlations, thus supporting the assumption of a specific pathway between FAA and ASD traits. Further studies are needed in this direction.

## 5. Conclusions

These findings support the use of objective measurements of EEG frontal alpha asymmetry to delineate specific pathophysiological mechanisms in ASD. Notably, this study reports a prediction of ASD symptoms at age 24 months. However, our longitudinal data collection is ongoing, and we are prospectively following our current cohort to identify children who will or will not receive a diagnosis of ASD. Characterization of reliable biomarkers will guide the detection of the most vulnerable infants that will benefit from early intervention and rehabilitation, with the long-term aim of substantially reducing the heavy impact of ASD on the National Health System.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-3425/9/12/342/s1, Figure S1: Sensor layout of the 60-channel Hydro-Cel Geodesic Sensor Net used in the study. Red and blue squares represent the electrodes included, respectively, in the Left and Right frontal clusters and entered into statistical analyses (see Gabard-Durnam et al., 2015).

**Author Contributions:** V.R. planned the study, performed data analyses and prepared the manuscript; C.M. performed data analyses, supervised the manuscript; C.P. supervised the experiment, performed data analysis; E.M.R. and G.M. were involved in participant recruitment, data collection and processing; M.M. supervised the manuscript; C.C. supervised the experiment, performed data analyses and prepared the manuscript.

**Funding:** This study was funded by the Italian Ministry of Health (Ricerca Corrente "2016, 2017, 2018, 2019" to Chiara Cantiani, Ricerca Finalizzata "NET-2013-02355263-2") and by "5 per mille" funds for biomedical research to Valentina Riva.

**Acknowledgments:** The authors wish to thank all participating infants and their parents.

**Conflicts of Interest:** The authors declare no conflict of interest.

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brain sciences

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*Review*

# Cannabinoids for People with ASD: A Systematic Review of Published and Ongoing Studies

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Received: 15 July 2020; Accepted: 17 August 2020; Published: 20 August 2020

**Abstract:** The etiopathogenesis of autism spectrum disorder (ASD) remains largely unclear. Among other biological hypotheses, researchers have evidenced an imbalance in the endocannabinoid (eCB) system, which regulates some functions typically impaired in ASD, such as emotional responses and social interaction. Additionally, cannabidiol (CBD), the non-intoxicating component of *Cannabis sativa*, was recently approved for treatment-resistant epilepsy. Epilepsy represents a common medical condition in people with ASD. Additionally, the two conditions share some neuropathological mechanisms, particularly GABAergic dysfunctions. Hence, it was hypothesized that cannabinoids could be useful in improving ASD symptoms. Our systematic review was conducted according to the PRISMA guidelines and aimed to summarize the literature regarding the use of cannabinoids in ASD. After searching in Web of Knowledge$^{TM}$, PsycINFO, and Embase, we included ten studies (eight papers and two abstracts). Four ongoing trials were retrieved in ClinicalTrials.gov. The findings were promising, as cannabinoids appeared to improve some ASD-associated symptoms, such as problem behaviors, sleep problems, and hyperactivity, with limited cardiac and metabolic side effects. Conversely, the knowledge of their effects on ASD core symptoms is scarce. Interestingly, cannabinoids generally allowed to reduce the number of prescribed medications and decreased the frequency of seizures in patients with comorbid epilepsy. Mechanisms of action could be linked to the excitatory/inhibitory imbalance found in people with ASD. However, further trials with better characterization and homogenization of samples, and well-defined outcomes should be implemented.

**Keywords:** autism spectrum disorder; cannabinoids; cannabidiol; cannabidivarin; THC; problem behaviors; sleep; epilepsy; hyperactivity; side effects

## 1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in communication and social interaction and by a pattern of restricted interests and repetitive behaviors that might vary in severity [1]. It was estimated that around 1.5% of the general population might belong to the autism spectrum [2]. Along with core symptoms, ASD might present with several associated problems, such as irritability, challenging behaviors [3], and self-injury [4], especially in the presence of associated intellectual disability (ID), a condition that seemed to regard at least one-third of the autistic population [5]. Conversely, individuals with higher cognitive abilities are more frequently

burdened by psychiatric comorbidities, such as depression, anxiety, attention deficit-hyperactivity disorder (ADHD), or sleep problems [6–8]. Medical comorbidities are also highly prevalent among the ASD population [9–11]. In particular, epilepsy represents the most frequent co-occurring neurological condition, affecting 5 to 30% of individuals with ASD [12–15]. Even in absence of frank seizures, people with ASD seem to present subclinical electrical discharges with abnormalities in EEG patterns [16,17].

The etiopathogenesis of ASD still needs to be clarified. Several genetic [18], perinatal [19,20], and environmental factors [21,22] seem to be involved. Research has also evidenced an imbalance in some endogenous neurotransmission systems [23], such as the serotoninergic [24], γ-aminobutyric acid (GABA)-ergic [17,25], and endocannabinoid (eCB) system [26–28].

Imbalances in the eCB neurotransmission system were found in animal models of ASD [29]. Additionally, lower serum levels of eCB were detected in children with ASD compared to typically developing peers [30,31]. Notably, the eCB system is relevant, as it seems to regulate some of the functions typically impaired in ASD, such as the form of emotional responses and social interaction [32].

Given the alterations in the eCB systems, researchers started to hypothesize that phytocannabinoids, which are naturally present in the plant of *Cannabis sativa*, might exert beneficial effects on the core and associated symptoms of ASD. First, multiple experimental studies conducted on mouse models showed that cannabidiol (CBD), the non-intoxicating component of cannabis, affects social interaction [33,34], which is severely impaired in ASD. Although CBD does not exert psych mimetic properties or the ability to induce addiction, it indirectly affects the transmission of the cannabinoid-related signal, the degradation of the endocannabinoid anandamide, and thus act on autistic-like symptoms in rats [35].

Interestingly, in June 2019, the US Food and Drug Administration (FDA) approved the Epidyolex, a CBD-based oral solution, for the treatment of seizures in Dravet and Lennox-Gastaut syndrome, two rare forms of epilepsy, in children older than two years of age [36]. As mentioned above, epilepsy is one of the most frequent co-occurring conditions of ASD, and the presence of seizures or non-epileptic abnormalities in EEG patterns might be partially responsible for the challenging behaviors or aggression in people with ASD. Thus, the correction of these abnormalities could improve, at least in part, the behavioral problems [37]. Moreover, the common co-existence of ASD and epilepsy suggests the presence of shared neuropathological mechanisms. Of note, both conditions are associated with abnormalities in the inhibitory GABA neurotransmission, including reduced $GABA_A$ and $GABA_B$ subunit expression. These abnormalities can elevate the excitatory/inhibitory balance, resulting in a hyper-excitability of the cortex, with an increased risk of seizures [38]. The literature showed that CBD seems to act similarly to antiepileptic drugs, as it increases the GABA transmission, thus reducing neuronal excitability [39,40].

Additionally, CBD exerts an agonist activity on the 5-HT1a receptors (i.e., serotoninergic system), which could mediate its pharmacological antidepressant, anxiolytic, and pro-cognitive properties [41,42]. In fact, the therapeutic effects of CBD were tested in patients suffering from anxiety disorder [43], a psychiatric comorbidity affecting at least 20% of people with ASD [8]. Possible benefits of CBD, due to its potential effects on the dopaminergic system, were also studied on subjects suffering from psychosis, [44], which could also represent a mental health issue for autistic individuals [8].

The effects of other cannabinoids were scarcely explored in clinical research. Cannabidivarin (CBDV) improved neurological and social deficits in early symptomatic Mecp2 mutant mice, a model of the Rett syndrome [45]. Moreover, it was proven to be an effective anticonvulsant in several models of epilepsy [46]. Delta-9-tetrahydrocannabinol (THC), the psychoactive component of cannabis, might increase sleep duration [47], thus being a potential candidate for a sedative effect. Additionally, it seems to reduce locomotor activity, which is indicative of a decrease in anxiety-like behavior [48]. According to a recent pilot randomized trial [49], a cannabinoid compound containing a 1:1 ratio of THC:CBD, significantly improved symptoms of hyperactivity, impulsivity, and inhibition measures in adults with ADHD, a condition that seemed to affect around 28% of autistic subjects [8].

As mentioned above, ASD presents serious deficits in social interaction and communication, as well as repetitive behaviors. However, till date, no effective pharmacological treatment exists for ASD core symptoms; only two atypical antipsychotics (i.e., risperidone and aripiprazole) were approved by the FDA for the treatment of irritability in children and adolescents with ASD [50]. Nevertheless, psychotropic medications are frequently prescribed in everyday clinical practice, with the frequent onset of side effects [51]. Given their properties, cannabinoids were proposed as candidate therapeutic options in people with ASD. Two recent narrative reviews were conducted on the topic [52,53]. However, to the best of our knowledge, no systematic reviews have comprehensively summarized the effects of cannabinoids for the treatment of individuals with ASD. The present paper aimed to describe the current state-of-the-art regarding the use of cannabinoids in individuals with ASD, focusing on both published and ongoing trials.

## 2. Materials and Methods

### 2.1. Search Strategy

We followed the PRISMA Statement guidelines to perform a systematic search [54]. First, we searched the following databases from inception up to 26 May 2020: Web of Knowledge™ (including Web of Science, MEDLINE®, KCI—Korean Journal Database, Russian Science Citation Index, and SciELO Citation Index), PsycINFO, Embase, and ClinicalTrials.gov, without any time or language restriction. We used the following search strategy: *(cannab *) AND (autis * OR asperger OR kanner OR "neurodevelop * disorder *").* Second, we reviewed all references of relevant reviews and meta-analyses to find any additional eligible study.

### 2.2. Eligibility Criteria

Two review authors (LF and VC) screened all retrieved papers, independently and in duplicate. Any doubt was solved by consensus. The authors included all original studies written in English, published as full papers or abstracts in peer-reviewed journals, and met the following criteria:

(1) Participants: Individuals with a diagnosis of autism spectrum disorder (ASD), according to international valid criteria or measured by a validated scale, regardless of age.

(2) Intervention: *Cannabis sativa* or cannabinoids, such as, cannabidiol (CBD), cannabidivarin (CBDV), delta-9-tetrahydrocannabinol (THC) and others, administered at any dosage and any form.

(3) Comparison: Studies with or without a comparison group (placebo or other forms of treatment).

(4) Outcomes: Any outcome.

(5) Study design: Case report, case series, retrospective, observational longitudinal, randomized or controlled clinical trials, both parallel and crossover.

### 2.3. Data Extraction

Data were extracted by two authors (S.T. and I.C.) who worked independently and in duplicate. Any doubt was solved by consensus. A standardized form was used to extract data from the included studies. We extracted information about study characteristics (authors, year, study design, country), characteristics of the ASD sample (sample size, age, presence of ID, presence of epilepsy, concomitant medications), type and duration of the intervention and the comparison, outcomes and outcome measures, findings, and side effects. We also reported data regarding ongoing studies, as retrieved in ClinicalTrials.gov. Results of the study were reported in a narrative summary that was organized around the study characteristics.

## 3. Results

### *3.1. Search Results*

Our search yielded a total of 758 studies, while four additional articles were found through other sources. After removing duplicates, we screened 604 titles and abstracts. After reading the full texts of 24 papers, we finally included 10 published works (eight full articles and two conference abstracts) in our systematic review. Additionally, nine ongoing trials were found in ClinicalTrial.gov, of which four met the eligibility criteria. The selection procedure of the included studies was reported in Figure 1.

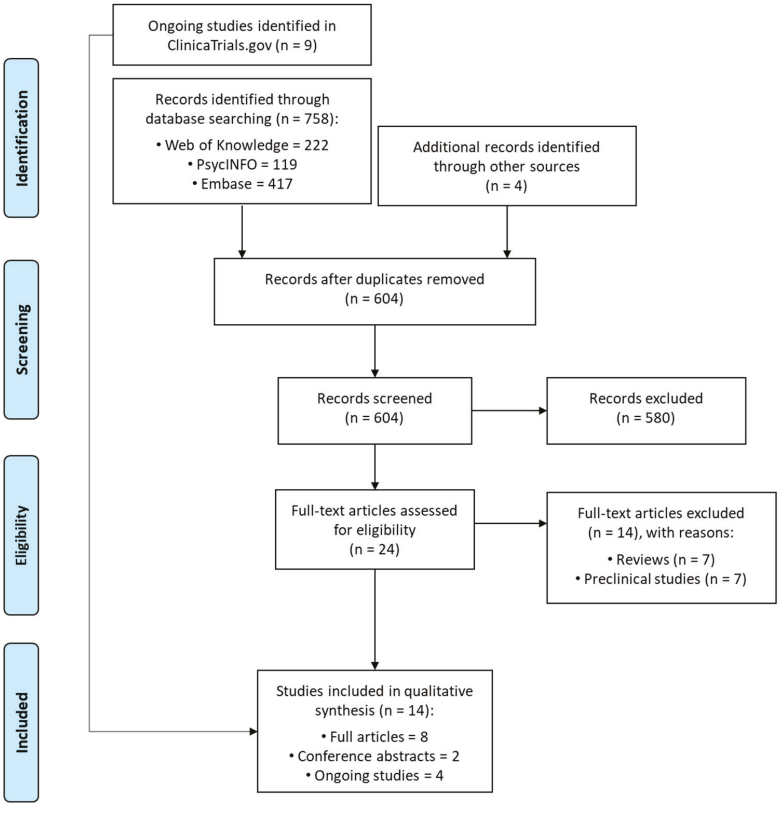


**Figure 1.** PRISMA flow chart of the study selection process.

### *3.2. Characteristics of Studies and Participants*

We included three retrospective studies, three prospective studies, one case report, and three randomized placebo-controlled crossover trials. Apart from the case report [55], all papers were published within the last three years. Studies were conducted in Israel ($n$ = 3), United Kingdom ($n$ = 3), Brazil, Chile, Austria, and United States ($n$ = 1 each). Sample sizes ranged from one [55] to 188 [56]. Participants were mainly children, although in two studies there were mixed samples [57,58]. The three studies conducted by Pretzsch and colleagues [59–61] included only adults with normal cognitive abilities (IQ > 70). Interestingly, only another study [62] specified the level of functioning, which was not reported in the remaining papers. Many participants were taking concomitant medications, and part of the samples had epilepsy. However, this information was not specified in two studies [55,57]. Study characteristics are reported in Table 1.

**Table 1.** Characteristics of the included studies.

| Study Characteristics | | | | Characteristics of Participants with ASD | | | | | Treatment Characteristics | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Authors** | **Year** | **Country** | **Study Design** | **N of Participants with ASD** | **Mean Age, Years (Range)** | **Intellectual Disability, *n* (%)** | **Concomitant Medication** | **Participants with Epilepsy, *n* (%)** | **Active Treatment** | **Daily Dosage** | **Control Treatment** | **Mean Follow-Up, Months (Range)** |
| Aran et al. [62] | 2019 | Israel | Retrospective cohort study | 60 | 11.8 (5–17.5) | 77% low functioning | All medications (82%), Antipsychotics (72%), Mood stabilizers (17%), Benzodiazepines (12%), SSRI (7%), Stimulants (7%) | 14 (23.3) | Cannabinoid oil solution at a 20:1 ratio of CBD and THC, | Sublingual assumption 2 or 3 times/daily with CBD doses started at 1 mg/kg/day and titrated up to 10 mg/kg/day. | None | 10.9 (7–13) |
| Barchel et al. [57] | 2019 | Israel | Prospective cohort study | 53 | 11 (4–22) | Not reported | Atypical antipsychotics (58.4%), Anti-epileptic (15%), Typical antipsychotics (11.3%), Stimulants (9.4%), Melatonin (7.5%), Other anti-muscarinic (5.6%), Anti-depressant (3.7%), Alpha agonist (1.8%) | Not reported | Cannabinoid oil solution at a concentration of 30% and 20:1 ratio of CBD and THC. | CBD: 16 mg/kg (maximal daily dose 600 mg), THC: 0.8 mg/kg (maximal daily dose of 40 mg). | None | Median 66 days (30–588 days) |
| Fleury-Teixeira et al. [63] | 2019 | Brazil | Prospective cohort study | 18 (15 analyzed) | 10.9 (6–17) | Not reported | Any medication (66.7%), Antipsychotics (46.7%), Mood stabilizers (33%), Phenobarbital (6.7%) | 5 (27.7) | *Cannabis Sativa* extract containing a 75:1 CBD:THC ratio | CBD: mean 175 mg/day (100–350); THC: 2.33 mg/day (1.33–2.33). | None | 12.4 (6–39) |
| Kuester et al. [58] | 2017 | Chile | Retrospective case series (abstract only) | 20 | 9.8 (2–22) | Not reported | Not reported | Part of the sample had seizures | 71.5% of patients received balanced CBD:THC extracts; 19% high-CBD; and 9.5% high-THC extracts. | Not reported | None | 7.6 (3–12) |

**Table 1.** *Cont.*

| Study Characteristics | | | | Characteristics of Participants with ASD | | | | | Treatment Characteristics | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Authors** | **Year** | **Country** | **Study Design** | **N of Participants with ASD** | **Mean Age, Years (Range)** | **Intellectual Disability, *n* (%)** | **Concomitant Medication** | **Participants with Epilepsy, *n* (%)** | **Active Treatment** | **Daily Dosage** | **Control Treatment** | **Mean Follow-Up, Months (Range)** |
| Kurz and Blaas [55] | 2010 | Austria | Case report | One | 6 | Not reported | None | Not reported | Dronabinol (delta-9-THC) solved in sesame oil. | Initial dosage was one drop (0.62 mg) in the morning which was increased up to 3.62 mg/die | None | 6 |
| McVige et al. [64] | 2020 | United States | Retrospective case series (abstract only) | 20 | Not reported | Not reported | Each patient tried an average of 6.4 other medications. Current medication not specified. | 6 (30%) | Medical cannabis | Not reported | None | Not reported |
| Schleider et al. [56] | 2019 | Israel | Prospective cohort study | 188 | 12.9 (<5–18) | Not reported | Antipsychotics (56.9%), antiepileptics (26.0%), hypnotics and sedatives (14.9%), antidepressants (10.6%). | 27 (14.4%) | Most patients consumed oil with 30% CBD and 1.5% THC. Insomnia was treated with an evening dose of 3% THC oil. | On average 79.5 ± 61.5 mg CBD and 4.0 ± 3.0 mg THC, three times a day. Average additional 5.0 ± 4.5 mg THC daily for insomnia. | None | 6 |
| Pretzsch et al. [59] | 2019a | United Kingdom | RCT crossover | 17 | 31.3 | 0 (0) | No medication influencing GABA+ levels. Methylphenidate ($n$ = 1), sertraline ($n$ = 1) | 0 (0) | CBD | 600 mg | Placebo | Single administration |
| Pretzsch et al. [60] | 2019b | United Kingdom | RCT crossover | 17 | 31.3 | 0 (0) | No medication influencing GABA+ levels. | 0 (0) | CBDV | 600 mg | Placebo | Single administration |
| Pretzsch et al. [61] | 2019c | United Kingdom | RCT crossover | 13 | 30.8 | 0 (0) | No medication influencing GABA+ levels. Methylphenidate ($n$ = 1), sertraline ($n$ = 1) | 0 (0) | CBD | 600 mg | Placebo | Single administration |

Legend: *CBD*: Cannabidiol; *CBDV*: Cannabidivarin; *GABA+*: Gamma aminobutyric acid; *RCT*: randomized controlled trial; *THC*: delta-9-tetrahydrocannabinol.

### *3.3. Characteristics of Treatment*

The treatment was represented by a cannabinoid oil solution with a CBD:THC ratio of 20:1 in two studies [57,62] and with a 30:1.5 ratio in one study [56]. Fleury-Teixeira et al. [63] and Kuester et al. [58], instead used *Cannabis sativa* extracts with different compositions. Kurz and Blaas [55] reported the use of dronabinol (delta-9-THC) dissolved in sesame oil. McVige et al. [64] documented the use of medical cannabis with unspecified composition. Finally, Pretzsch and colleagues administered single doses of 600 mg of CBD or CBDV [59–61]. Only the studies by Pretzsch et al. used a control treatment (placebo). The duration of treatment was extremely variable, ranging from single administrations [59–61] to six months [55,56]. Of note, many studies were naturalistic and treatment duration was different among participants. Characteristics of treatment with cannabinoids are reported in Table 1.

### *3.4. Outcomes and Findings*

The results of the included studies are reported in Table 2. It could be observed that studies typically had multiple outcomes. The most investigated were global impression, sleep problems, hyperactivity, problem behaviors, use of concomitant medications, and seizures. Parenting stress was measured in two studies [58,62]. Anxiety, mood, and quality of life were evaluated in the context of global impression. Only one study [63] specifically evaluated socio-communication impairments, reporting a median perceived improvement of 25%. However, the authors did not use standardized tools to measure the changes in the communication and social interaction domain. Surprisingly, none of the included studies aimed to evaluate changes in stereotypies. Of note, the three studies conducted by Pretzsch et al. [59–61] investigated the acute effects of cannabinoids using neuroimaging techniques (magnetic resonance spectroscopy [MRS] and functional Magnetic Resonance Imaging [fMRI]). Outcomes and results are reported in Table 2.

### *3.5. Ongoing Trials*

We retrieved four ongoing studies from ClinicalTrials.gov, of which two were randomized controlled trials and two open label trials. Three of these studies are being conducted in the United States, and one in Israel. Researchers mainly planned to recruit children (except for the trial NCT02956226, which planned to extend the administration of treatment up to the age of 21 years). Two studies are testing the effects of CBDV, one study is examining the effects of CBD at different dosages, and one is looking at the effects of a combination of CBD and THC (ratio 20:1). Duration of trials are from 6 to 52 weeks. All trials planned to administer multiple outcome measures to both patients and caregivers. Interestingly, specific tools measuring changes in ASD core symptoms were inserted, including the evaluation of repetitive behaviors and stereotypies. Adaptive abilities, aberrant behaviors, and sleep disturbances are other target symptoms of the studies. The characteristics of the ongoing trials are summarized in Table 3.

**Table 2.** Efficacy and safety of cannabinoids in people with autism spectrum disorder (ASD).

| Authors | Year | Outcome (Measures) | Results | Side Effects (%) | Drop Out/Treatment Discontinuation, *n* (%) |
|---|---|---|---|---|---|
| Aran et al. [62] | 2019 | - Problem behaviors (HSQ)<br>- Parenting stress (APSI)<br>- Caregiver Global Impression (CaGI): anxiety, behavior, communication<br>- Medication | - HSQ: improved by 29%.<br>- APSI: improved by 33%.<br>- CaGI-C: Behavior improved in 61%; anxiety improved in 39%; communication improved in 47% of the children.<br>- Following the cannabis treatment, 33% received fewer medications or lower dosage, 24% stopped taking medications and 8% received more medications or higher dose. | Any adverse event (51%), Sleep disturbances (14%), Restlessness (9%), Nervousness (9%), Loss of appetite (9%), Gastrointestinal symptoms (7%), Unexplained laugh (7%), Mood changes (5%), Fatigue (5%), Nocturnal enuresis (3.5%), Gain of appetite (3.5%), Weight loss (3.5%), Weight gain (3.5%), Dry mouth (3.5%), Tremor (3.5%), Sleepiness (2%), Anxiety (2%), Confusion (2%), Cough (2%), Psychotic event (2%) | 1 (1.6%) |
| Barchel et al. [57] | 2019 | - Hyperactivity<br>- Self-injury<br>- Sleep<br>- Anxiety<br>- Global improvement | - Hyperactivity: Improvement: 68.4%; No change: 28.9%; Worsening: 2.6%<br>- Self-Injury: Improvement: 67.6%; No change: 23.5%; Worsening: 8.8%<br>- Sleep Problems: Improvement: 71.4%; No change: 23.8%; Worsening: 4.7%<br>- Anxiety: Improvement: 47.1%; No change: 29.4%; Worsening: 23.5%<br>- Overall: Improvement: 74.5%; No change: 21.6%; Worsening: 3.9% | Somnolence (22.6%), Appetite decrease (11.3%), Appetite increase (7.5%), Insomnia (3.7%), Sense abnormality response (to temperature) (3.7%), Eyes blinking (3.7%), Diarrhea (3.7%), Hair loss (1.8%), Nausea (1.8%), Confusion (1.8%), Acne (1.8%), Palpitations (1.8%), Urinary (1.8%), Incontinence (1.8%), Eye redness (1.8%), Constipation (1.8%) | 5 (9.4%) |
| Fleury-Teixeira et al. [63] | 2019 | - Attention Deficit/Hyperactivity Disorder (ADHD)<br>- Behavioral disorders (BD)<br>- Motor deficits (MD)<br>- Autonomy deficits (AD)<br>- Communication and social interaction deficits (CSID)<br>- Cognitive Deficits (CD)<br>- Sleep Disorders (SD)<br>- Seizures (SZ)<br>- Concomitant medication | - ADHD: median perception of improvement: 30%<br>- BD: median perception of improvement 20%<br>- MD: median perception of improvement 20%<br>- AD: median perception of improvement 10%<br>- CSID: median perception of improvement 25%<br>- CD: median perception of improvement 20%<br>- SD: median perception of improvement 40%<br>- SZ: three participants reported ≥50% of improvement; two participants reported 100% of improvement<br>- Concomitant medication: complete withdrawal ($n$ = 3), partial withdrawal ($n$ = 1), partial withdrawal + dosage reduction ($n$ = 3), dosage reduction ($n$ = 2), no changes in medication use ($n$ = 1) | Sleepiness, moderate irritability ($n$ = 3); diarrhea, increased appetite, conjunctival hyperemia, and increased body temperature ($n$ = 1). All these side effects were mild and/or transient. Nocturia ($n$ = 2), which in one case appeared concomitantly to an improvement in sleep quality. | 3 out of 18 (16.7%) |

**Table 2.** *Cont.*

| Authors | Year | Outcome (Measures) | Results | Side Effects (%) | Drop Out/Treatment Discontinuation, *n* (%) |
|---|---|---|---|---|---|
| Kuester et al. [58] | 2017 | - Global Impression (CGI-I)<br>- Parenting stress (APSI)<br>- Other variables (sensory difficulties, food acceptance, sleep, seizures) | CGI-I And APSI: 66.7% of patients had significant improvement. Most cases improved at least one of ASD core symptoms. Sensory difficulties, food acceptance, feeding and sleep disorders, and/or seizures were improved in most cases. | Two patients had more agitation and one had more irritability, effects that were solved by changing the strain. | None |
| Kurz and Blaas [55] | 2010 | - Problem behaviors (ABC) | Significant improvement in all subscales | None reported. | None |
| McVige et al. [64] | 2020 | - Caregiver Global Impression (CaGI), including quality of life (QoL), activity limitations, symptoms, mood.<br>- Epilepsy<br>- Pain<br>- Other variables: sleep, aggression, communication, attention<br>- Medication use | - CaGI: improvement in all areas: QoL, activity limitations, symptoms, and mood<br>- Improvement in seizure frequency and severity<br>- Improvement in degree of overall pain<br>- Improvement in sleep, mood, aggression towards self and/or others, communication abilities and attention/concentration<br>- 50% of patients discontinued or reduced the use of other medications | Three patients reported mild adverse events (unspecified). | None |
| Schleider et al. [56] | 2019 | - Quality of life<br>- Mood<br>- Adaptive abilities<br>- Sleep<br>- Concentration<br>- Symptom change: Restlessness, Rage attacks, Agitation, Sleep problems, Speech Impairment, Cognitive impairment, Anxiety, Incontinence, Seizures, Limited Mobility, Constipation, Tics, Digestion Problems, Increased Appetite, Lack of Appetite, Depression | - Quality of life: 66.8% improvement<br>- Mood: 63.5% improvement<br>- Adaptive abilities: 42.9% improvement<br>- Sleep: 24.7% improvement<br>- Concentration: 14% improvement | Restlessness (6.6%), sleepiness (3.2%), psychoactive effect (3.2%), increased appetite (3.2%), digestion problems (3.2%), dry mouth (2.2%), lack of appetite (2.2%). | 23 (12.2%) |

**Table 2.** *Cont.*

| Authors | Year | Outcome (Measures) | Results | Side Effects (%) | Drop Out/Treatment Discontinuation, *n* (%) |
|---|---|---|---|---|---|
| Pretzsch et al. [59] | 2019a | MRS, effects of Glx and GABA+ | CBD increased subcortical, but decreased cortical, Glx. CBD decreased GABA+ in ASD. | None reported | None |
| Pretzsch et al. [60] | 2019b | MRS, effects of Glx and GABA+ | CBDV significantly increased Glx in the basal ganglia. In the ASD group, the 'shift' in Glx correlated negatively with baseline Glx concentration, CBDV had no significant impact on Glx in the DMPFC, or on GABA+. | None reported | None |
| Pretzsch et al. [61] | 2019c | fMRI, measure of fractional amplitude of low-frequency fluctuations' (fALFF) and, functional connectivity (FC) | CBD significantly increased fALFF in the cerebellar vermis and the right fusiform gyrus in the ASD group. CBD also significantly altered vermal FC with several of its subcortical (striatal) and cortical targets but did not affect fusiform FC with other regions. | None reported | None |

Legend: *ABC*: Aberrant Behavior Checklist; *APSI*: Autism Parenting Stress Index; *CBD*: Cannabidiol; *CBDV*: Cannabidivarin; *CGI-I*: Clinical Global Impression-Improvement; *fMRI*: functional Magnetic Resonance Imaging; *GABA+*: Gamma aminobutyric acid; *Glx*: glutamate + glutamine; *HSQ*: Home situation Questionnaire; *MRS*: Magnetic Resonance Spectroscopy.

**Table 3.** Characteristics of ongoing trials testing cannabinoids in people with ASD.

| Study Characteristics | | | | | Participants Characteristics | | Treatment Characteristics | | | Outcomes |
|---|---|---|---|---|---|---|---|---|---|---|
| **Registration Number** | **Principal Investigator** | **Affiliation** | **Country** | **Study Design** | **N of Participants with ASD** | **Age Range** | **Active Treatment** | **Control Treatment** | **Duration** | **Outcome Measures** |
| NCT03202303 | Eric Hollander | Montefiore Medical Center | United States | RCT | 100 | 5–18 | 10 mg/kg/day CBDV | 10 mg/kg/day placebo | 12 weeks | ABC-I; RBS-R; ABC-SW; PedsQL; Vineland 3; CGI-I |
| NCT03849456 | Gregory N Barnes | University of Louiseville | United States | Open label | 30 | 4–18 | CBDV at a dose of 2.5 mg/kg/day and titrate to a target dose of 10 mg/kg/day or 800 mg/day during the first 4 week. If intolerance during titration, participant may be maintained on a dose below 10 mg/kg/day. Maximum dose: 20 mg/kg/day or 1600 mg/day. | None | 52 weeks | TEAEs; CCC-2; Vineland 3; NIH; RBS-R; CSHQ; ABC, CGI-I |
| NCT03900923 | Francisco Castellanos, Orrin Devinsky | New York Langone Health | United States | Open label | 30 | 7–17 | Cohorts of size 3 receiving doses of 3, 6, or 9 mg/kg/day of CBD, depending on the treatment response of participants in prior cohorts. | None | 6 weeks | CGI-I; BOSCC; RBS-R; SRS-2; ABC-SW; ABC-I; CCC-2; SCARED; SDSC; Vineland 3; CGI-S; AFEQ; ASC-ASD-P; ASC-ASD-C; OSUS; OSUI; BIS |
| NCT02956226 | Adi Aran, Varda Gross | Shaare Zedek Medical Center | Israel | RCT | 150 | 5–21 | Oral cannabinoids mix (CBD:THC in a 20:1 ratio) at 1 mg/kg CBD per day, up titrated until intolerance or to a maximum dose of 10 mg/kg CBD per day, divided to 3 daily doses. | Oral olive oil and flavors that mimic in texture and flavor the cannabinoids' solution. | 3 months | HSQ-ASD; CGI-I; SRS-2; APSI; LAEP |

**Legend**: *ABC*: Aberrant Behavior Checklist; *ABC-I*: Aberrant Behavior Checklist-Irritability Subscale; *ABC-SW*: Aberrant Behavior Checklist-Social Withdrawal Subscale; *AFEQ*: Autism Family Experience Questionnaire; *APSI*: Autism Parenting Stress Index; *ASC-ASD-C*: Anxiety Scale for Children—Autism Spectrum Disorder—Child Versions; *ASC-ASD-P*: Anxiety Scale for Children—Autism Spectrum Disorder—Parent Versions; *BIS*: Behavioral Inflexibility Scale; *BOSCC*: Brief Observation of Social Communication—Change; *CBD*: Cannabidiol; *CBDV*: Cannabidivarin; *CCC-2*: Change from Baseline in Children's Communication Checklist-2; *CGI-I*: Clinical Global Impressions-Improvement; *CGI-S*: Clinical Global Impression-Severity; *CSHQ*: Change from Baseline in Children's Sleep Habits Questionnaire; *HSQ-ASD*: Home Situations Questionnaire-Autism Spectrum Disorder; *LAEP*: Modified Liverpool Adverse Events Profile; *NIH*: Change from Baseline in National Institutes of Health; *OSUI*: Autism Clinical Global Impressions: Improvement; *OSUS*: OSU Autism Clinical Global Impressions: Severity; *PedsQL*: Pediatric Quality of Life Inventory—Family Impact Module; *RBS-R*: Repetitive Behavior Scale-Revised; *RCT*: randomized controlled trial; *SCARED*: Screen for Child Anxiety Related Disorders; *SDSC*: Sleep Disturbance Scale for Children; *SRS-2*: Social Responsiveness Scale, 2nd Edition; *TEAEs*: Number of Participants Who Experienced Severe Treatment-Emergent Adverse Events; *THC*: delta-9-tetrahydrocannabinol; *Vineland 3*: Vineland Adaptive Behavior Scale-3.

## 4. Discussion

In the present systematic review, we found preliminary evidence showing that cannabinoids (compounds with different ratios of CBD and THC), might exert beneficial effects on some ASD-associated symptoms, such as behavioral problems, hyperactivity, and sleep disorders, with a lower number of metabolic and neurological side effects than medications. Importantly, treatment with cannabinoids allowed to reduce the number of prescribed medication and significantly reduced the frequency of seizures in participants with comorbid epilepsy. We will now reflect in-depth on some critical points related to the main findings, mechanisms of action of cannabinoids, and methodology of the included studies.

### *4.1. Efficacy and Safety of Cannabinoids in ASD*

The majority of available interventions for ASD are based on behavioral, psychoeducational, and pharmacological therapies [65]. To date, the FDA has approved only two medications for the treatment of children and adolescents with ASD—risperidone and aripiprazole. Such medications are mostly used for irritability, aggressiveness, and self-injurious behaviors, but, unfortunately, there is no evidence of efficacy on core symptoms [66]. However, many drugs, such as antipsychotics, mood stabilizers, antidepressants, and stimulants, are prescribed off-label in clinical practice [51,67].

The findings of the studies included in the present systematic review are promising, as cannabinoids seem to improve some associated symptoms in many individuals with ASD, such as behavioral problems, hyperactivity, and sleep disorders. On the contrary, changes in core symptoms were scarcely explored—only one study [63] reported some improvements in communication and social interaction in a small sample of Brazilian children with ASD. No studies specifically investigated the effect of cannabinoids on repetitive behaviors or restricted interests. Of note, in individuals with comorbid epilepsy, the use of cannabinoids significantly reduced the frequency and intensity of seizures. Additionally, the number and dosage of used medications were reduced after the treatment with cannabinoids. This is a secondary, but extremely important finding. In fact, pharmacological therapies commonly prescribed to individuals with ASD are frequently burdened by side effects, such as weight gain, dyslipidemia, diabetes, and metabolic syndrome. These adverse events are also frequent in children, given the sensory difficulties, food selectivity, and rigidity in eating behaviors, which can lead to an increased risk for weight gain and poor nutritional habits [68–71]. For this reason, the correct management of pharmacological treatment should try to prevent the onset of side effects, through reviewing and identifying the risk factors, monitoring metabolic markers, and promoting potential modifiers of the course of metabolic syndrome (i.e., lifestyle, polypharmacy) [72]. For example, patients with a history of weight or diabetes might avoid medications that are known to increase the risk of these side effects, such as risperidone and olanzapine [73,74]. Some cardiovascular risk factors (QTc prolongation, diabetes, and weight gain) also seem to have dose-dependent side effect profiles that might require monitoring at dose changes [68,74,75].

We found that the most common side effects of cannabinoids were somnolence, increased appetite, and irritability. As many patients were taking concomitant medications, it is not possible to determine if these adverse events were caused by the cannabinoids or by other drugs. Only Aran et al. [62] reported a severe adverse event (a psychotic episode) that resolved after stopping the cannabinoid oil solution and treating the patient with an antipsychotic (i.e., ziprasidone). None of the included studies reported cardiac adverse events (i.e., QTc prolongation) or severe metabolic side effects (i.e., hyperlipidemia, diabetes, hyperprolactinemia) that could depose for a better safety profile in cannabinoids than antipsychotics.

### *4.2. Mechanisms of Action: The Role of Excitatory/Inhibitory System*

The three papers published by Pretzsch et al. [59–61] primarily investigated the modulation of the brain's excitatory and inhibitory systems in adults with ASD and neurotypical controls, after a

single dose of 600 mg of cannabinoids (CBD and CBDV). The findings evidenced a CBD-related increase of glutamate (excitatory system) in subcortical regions (i.e., basal ganglia) and a decrease in cortical regions (i.e., dorso-medial prefrontal cortex), both in subjects with and without ASD. Conversely, CBD increased GABA transmission (inhibitory system) in critical and subcortical regions of neurotypical subjects, while decreased it in the same areas of the ASD group. This confirmed the hypothesis that GABA transmission could be altered in people with ASD [17,76,77]. Moreover, CBD modulated low-frequency activity, used as a measure of spontaneous regional brain activity, and functional connectivity in the brain of adults with ASD [61]. The experiment with CBDV replicated the findings of the CBD study for glutamate transmission, but not for GABA [60].

Such findings might further explain the link between autism and seizures. About 25% of children with treatment-resistant epilepsy are comorbid with ASD and often present other severe comorbidities, such as sleep disturbances, intellectual disability, or other psychiatric conditions [78]. Additionally, as mentioned above, epilepsy is one of the most frequent medical comorbidities in people with ASD [12–15], and is also found to be more common in those patients with autism-like behaviors as part of phenotypes of genetic syndromes (i.e., Angelman, Rett syndrome, etc.) [79]. This overlap might be explained by common biological mechanisms. Like ASD, in fact, epilepsy is characterized by an imbalance between excitatory and inhibitory transmission in the central nervous system [80]. The presence of seizure in ASD could also be responsible for the onset of challenging behaviors [81]. Therefore, it could be hypothesized that treating seizures with cannabinoids might also exert a significant impact on externalizing symptoms.

Unfortunately, the action of cannabinoid administration on other neurotransmission systems was not investigated in autistic individuals. As mentioned in the introduction, studies on animal models provided evidence for the role of serotoninergic [42,82,83] and dopaminergic systems [84]. However, their role in the etiology of ASD still needs to be clarified.

### *4.3. Limitation: Heterogeneity of Studies*

The present systematic review included ten published studies (of which two conference abstracts) and four ongoing trials. Looking at Table 1, which summarizes the characteristics of the studies, it is possible to notice that the works conducted to the present date are highly mixed in terms of study design and participants. Some studies included both children and adults, other participants with and without epilepsy (which is not irrelevant, as cannabinoids act on the excitatory/inhibitory system, altered in epilepsy). Additionally, the intake of concomitant medications acting on the GABAergic system might represent a bias. Finally, the level of functioning or the intelligence quotient (IQ) was specified only in four studies [59–62]. The characterization of samples is fundamental as target symptoms might vary. Individuals with associated intellectual disability (ID) typically present more severe behavioral problems that could benefit from the use of cannabinoids. People with higher levels of functioning, instead, could present more frequently concurrent anxiety disorders. This is important because different target symptoms need different outcome measures.

Other caveats rely, in fact, on the heterogeneity of outcomes and administered treatment. It seems evident that the studies were mainly explorative and did not report a differentiation between primary and secondary outcomes. Moreover, measures were often multiple and combined both core and associated ASD symptoms (e.g., global impression). Standardized measures were used only in a few studies, and in some cases, the authors reported only the proportion of improvement for each symptom. This important issue confirms the findings of a recent systematic review of 406 clinical trials [85], which pointed out that the tools used in autism research are heterogeneous and non-specific. This fragmentation might significantly hamper the comparison between studies and the understanding of the real effectiveness of cannabinoids in the ASD population. In addition, the majority of studies used combinations of CBD and THC in different concentrations and ratios, even in the same study sample. It is indisputable that the dosage of cannabinoids needs to be calibrated on individual characteristics

(e.g., weight), but again, the use of different concentrations/ratios does not allow to compare studies and find the optimal therapeutic range.

Importantly, seven of the included studies did not have a control group. Only the three studies conducted by Pretzsch et al. [59–61] administered a control treatment (placebo), while also using a control group (healthy subjects). However, these studies principally aimed to explore the neural modifications induced by the assumption of CBD or CBDV in individuals with ASD, while also evaluating the differences with neurotypical subjects. Even if not directly designed to study the efficacy and safety of cannabinoids in ASD, the completion of similar studies appears fundamental as they might elucidate the neurochemical functioning of the autistic brain.

## 5. Conclusions

Our systematic review was the first to critically summarize the published and ongoing studies investigating the use of cannabinoids in the ASD population. Despite cannabinoids having shown promising effects on some ASD-associated problems (e.g., aberrant behaviors, sleep disorders, hyperactivity, seizures), their efficacy on core symptoms (i.e., socio-communication impairments, restricted interests, and stereotypies) remains largely unknown. The main limitation of the present paper is the absence of a statistical analysis of results that was hampered by the heterogeneity of study design, populations, type of cannabinoid, and particularly, outcomes, and measures. Future studies investigating the acute effects of cannabinoids in people with ASD on neurotransmitters levels could clarify the mechanisms of action of cannabinoids. Moreover, the comparison with healthy samples might clarify at least some aspects of the etiopathology of ASD and lay the ground for potential treatments for core and associated symptoms. Even if some clinical trials are ongoing, there is the need for further long-term studies, with homogeneous samples in terms of age, medication use, level of functioning, and presence/absence of seizures. Of great importance would be the choice of specific primary and secondary outcomes, focused on the cluster of symptoms that could benefit from the use of cannabinoids.

**Author Contributions:** Conceptualization, L.F.-P., V.C., S.T., and I.C.; methodology, L.F.-P.; investigation, L.F.-P., V.C., S.T., and I.C.; data curation, L.F.-P., V.C., S.T., and I.C.; writing—original draft preparation, L.F.-P., V.C., S.T., and I.C.; writing—review and editing, A.P., M.S.S., C.M.D.-C., and E.A.; supervision, A.P., M.S.S., C.M.D.-C., and E.A.; All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Review*

# Autistic-Like Features in Visually Impaired Children: A Review of Literature and Directions for Future Research

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Received: 9 July 2020; Accepted: 24 July 2020; Published: 1 August 2020

**Abstract:** There remains great interest in understanding the relationship between visual impairment (VI) and autism spectrum disorder (ASD) due to the extraordinarily high prevalence of ASD in blind and visually impaired children. The broad variability across individuals and assessment methodologies have made it difficult to understand whether autistic-like symptoms shown by some children with VI might reflect the influence of the visual deficit, or represent a primary neurodevelopmental condition that occurs independently of the VI itself. In the absence of a valid methodology adapted for the visually impaired population, diagnosis of ASD in children with VI is often based on non-objective clinical impression, with inconclusive prevalence data. In this review, we discuss the current state of knowledge and suggest directions for future research.

**Keywords:** autism spectrum disorder; autistic-like features; social-cognitive development; stereotypical behaviors; visual impairment

## 1. Introduction

Research into the presence of autistic-like features among blind children has a long history. Starting from a series of publications appearing in the 1960s and 1970s [1–7] (which considered that autistic-like behaviors showed by blind children were a possible consequence of the lack of visual experience on the development of self-image and self-representation), researchers and clinicians have increasingly reported commonalities between children with autism spectrum disorder (ASD) and those with visual impairment (VI), particularly with regard to social interaction and communication skills [8–12]. Restricted symbolic play, difficulties in social interaction with peers and imitation, echolalic speech, and increased stereotyped behavior have all been frequently reported in blind children [9,10,13,14]. Indeed, these behaviors resemble subjects with ASD and are often termed "blindisms" since they are explainable in the context of VI [15]. However, the similarity between these "blindisms" and "autistic-like" behaviors, coupled with the lack of ASD assessment tools specifically designed for blind and visually impaired children, complicates the diagnosis of ASD in these individuals. Finally, while the estimated prevalence of ASD among sighted children is between 1 and 2% in Europe [16], determining the prevalence in the visually impaired population still varies greatly, ranging from 2 up to 50% [12,17–19]. The underlying mechanisms related to autistic-like symptoms shown by some children with VI, as well as how certain visually impaired children are able to overcome these developmental challenges, remains poorly understood.

Reviewing available literature, it remains to be established whether ASD in VI is primarily a neurodevelopmental condition that occurs independently of the visual disorder (possibly with a common causal agent such as a genetic defect), or is secondary to the VI, and is more closely associated with the disruption of vision on early interactive experiences, or represents a combination of the two [20,21]. For many of the children who are blind and also display features of ASD, it is possible that their characteristics (while being representative of ASD), actually follow a different developmental pathway than those who have ASD and normal vision.

Referring to papers published between 1958 and 2020, the purpose of this review is to provide a discussion of these important, yet still controversial issues. Using two electronic databases (PubMed and Google Scholar), we included combinations of the following search terms: "autism", "autism spectrum disorder", "blindness", "sight loss", and "visual impairment". Citations identified from the automated search were manually verified for appropriateness.

The original search yielded 1613 documents, that were reduced to 921 following duplicate removal. Independent screening (by the first and second authors of this review) of the study titles and abstracts was carried out to identify studies that were most relevant to the aims of this review.

Articles were included for further inspection if they satisfied the following inclusion criteria: (1) explored the mechanisms that may explain the observed relationship between VI and ASD, taking into account the nature and role of contributing risk factors such as the severity of VI, type of blindness, age at onset, and other associated impairments; (2) discussed specific behavioral and neurocognitive traits in visually impaired compared to ASD children such as: joint attention, language, verbal and non-verbal communication, theory of mind, stereotypical behaviors; (3) described the approaches employed to assess ASD in visually impaired children, with specific attention given to the fact that the most common methods used for scoring autistic behaviors include several items which are directly dependent on visual abilities; (4) included participants between 0 and 18 years of age.

Articles were included (irrespective of the age range) if they added relevant information, as judged by the authors. Articles were excluded if they were focused on the prevalence and/or the type of ophthalmic problems in the ASD population or the characteristic of visual deficit in specific genetic/metabolic conditions which also presented autistic-like traits. This resulted in the exclusion of 821 papers that did not meet these inclusion criteria and lead to a final sample of 100 studies for the purposes of qualitative synthesis.

## 2. The Observed Relationship between Visual Impairment and ASD: Possible Underlying Mechanisms and Contributing Risk Factors

Since the first reported description by Keeler [22] of a co-occurrence between blindness and autistic behavior, various studies have focused on identifying specific types of ophthalmological disorders as potential organic etiological factors, suggesting the presence of common causal agent potentially independent from the VI itself [22–24]. Keeler [22] hypothesized that autistic behavior in children with "retrolental fibroplasia" (i.e., retinopathy of prematurity) resulted from a combination of brain damage, blindness, and emotional deprivation. Wing [25] listed several similarities between children with ASD and children with partial blindness and partial deafness caused by maternal rubella. Chase [24] found a gradient of autistic-like features in a group of 246 individuals with "retrolental fibroplasia", but none had a clear diagnosis of infantile ASD. The author also reported a strong relationship between autistic-like symptoms and neurological findings. Chess [23] assessed the behavioral data of 243 children with congenital rubella and reported that the common component accounting for ASD in these observed cases was brain damage. Rogers and Newhart-Larson [26] reported the presence of ASD in 5 preschool children with Leber's congenital amaurosis and compared these children to a control group with congenital blindness due to other causes and typical development, suggesting that cerebellar deficit in some patients with Leber's congenital amaurosis could provide a neurobiological basis for the behavioral similarities observed between these patients and sighted autistic individuals. Ek and colleagues [27] studied the relationship between blindness due to retinopathy of prematurity (ROP) and

ASD and concluded that an ASD diagnosis in blind children is likely to be mediated by brain damage or dysfunction. Fazzi and colleagues [28] submitted 24 children affected by Leber's congenital amaurosis to a modified version of the Childhood Autism Rating Scale (CARS) by excluding item VII about visual responsiveness [29]. According to their results, 20 children were found to be non-autistic, 4 presented with mild/moderate ASD, and no child was found to be severely autistic. Nearly every child presented some degree of restricted and stereotyped patterns of interest, adherence to specific routines or rituals, difficulties in adapting to environmental changes and showed dysfunctional relationships with other people or in their social and emotional responsiveness. Impaired verbal communication, a tendency for passiveness, and difficulties in using their bodies were also observed. In a prospective study, Garcia-Filion and colleagues [30] demonstrated that autistic-like features occurred with high frequency in children with mild to severe optic nerve hypoplasia (OHN). Since the study included children with various degrees of VI (including those with unilateral ONH), this supported the hypothesis that the autistic component could have a neurological basis, rather than being connected to the visual impairment itself. Similarly, Parr and colleagues [31] assessed the prevalence of social, communicative, and repetitive or restricted behavioral (SCRR) difficulties and defined clinical ASD in 83 children with ONH and/or septo-optic dysplasia (SOD), finding the presence of at least one SCRR difficulty in 58%. Thirty-four percent of the sample was clinically diagnosed with ASD. Moreover, SCRR difficulties and ASD were statistically higher in children with significant cognitive impairment and profound VI and there was no evidence that additional neuro-anatomical abnormalities were a further risk factor in the development of ASD. These data suggested the authors that ASD in children with OHN and/or SOD may arise through different mechanisms compared to the idiopathic ASD population.

Jutley-Neilson and colleagues [32] evaluated the occurrence of ASDs in 28 children with SOD and 14 with ONH. According to the previous study of Parr et al. [31], 33% of children with SOD and ONH received a clinical diagnosis of ASD. Using the Social Communication Questionnaire, 55% of the children met the cut-off threshold for further investigation to differentiate between ASDs and non-ASD (row scores ≥15) and 21% met the cut-off for further investigation to differentiate between ASD and autism (row scores ≥22). The authors identified the degree of visual loss and the severity of intellectual disability as good predictors for ASD, and recommended that children with SOD/ONH would benefit from routine ASDs screening. De Verdier et al. [33] described neurodevelopmental impairments in children with congenital or early infancy blindness born over a decade in Sweden; they found that ASD was one of the most common additional impairment (38% of these population) and that the prevalence was higher in children with ONH (70%), in children with ROP (58%), in children with microphthalmia/anophthalmia (44%), and in children with LCA (36%).

In a different perspective, some researchers [8,10] have suggested that focusing on the cause of blindness is irrelevant, emphasizing rather on the role of sensory deprivation and environmental risk factors in the emergence of autistic-like behaviors. Goodman and Minne [34] assessed 17 congenitally blind children (aged 4 to 11 years) without any additional impairment using the Autism Behavior Checklist [35]. The prevalence of ASD in this sample was 23.5% using a critical cut-off number of symptoms to determine diagnosis. In a study by Brown et al. [36], a prevalence of 20.8% was determined investigating 24 congenitally blind children without any neurological damage (aged 3 to 9 years) using the CARS [29]. Hobson and colleagues [8] found that nine congenitally blind children were similar in their range of clinical features with nine sighted autistic children (age- and verbal IQ-matched).

Regardless of the ophthalmological diagnosis, the potential vulnerability may partially be caused by early blindness and may not only be limited to a lack of vision, but also to severe and early damage to the visual system, threatening the development of mental and emotional processes that allow children to organize experiences and develop different areas of learning [37].

Data from healthy populations suggests that mutual influences between vision and emotion start at very early stages of information processing [38]. The brain regions involved in mental and emotional states include the prefrontal cortex, limbic structures, and the insula as well as visual

areas [39]. In particular, enhanced activation of the occipitoparietal regions (corresponding to the dorsal visual processing stream) has been reported during the emotional processing of visual stimuli [39,40]. Abnormal neuronal responses of these cortical regions, such as what could be expected in cerebral visual impairment, may contribute to an impairment in emotional recognition [41].

Recently, Fazzi et al. [19], among 214 children with cerebral causes and 59 with peripheral causes of vision impairment, found that ASD was more prevalent compared to a general population, and that the prevalence varied according to the type of visual disorder (2.8% for cerebral and 8.4% for peripheral visual impairment). Moreover, the presence of autistic symptoms was consistent with the diagnosis of ASD only in subjects with cerebral visual impairment, while in those with peripheral visual impairment, many symptoms related to visual loss overlapped with the clinical features of ASD, making clinical diagnosis more challenging.

Moreover, it is not clear why some children fail to progress, or even regress their communicative and cognitive skills. Mukaddes et al. [17] showed that individuals with blindness and ASD have greater neurological impairment and more severe visual impairment with respect to individuals with blindness only. This suggests that, regardless of the cause of blindness, brain damage remains an important contributing factor for the development of ASD. Certain investigators [42,43] have also described a phenomenon of serious developmental disruption or "setback" which seems to occur between the 15th and 27th month of age. An explanation for this setback occurring in children with profound visual impairment relates to the notion of a sensitive or critical period of brain development within the first to second year of life that relies on normal visual experience occurring within this period [43]. Finally, in a retrospective study by Waugh and colleagues [44], a higher proportion of brain lesions detected with magnetic resonance imaging (MRI) was associated with greater developmental setback in children with visual impairment, which may be an early manifestation of clinical ASD [42,43].

More recently, Vervoled et al. [45] reviewed the literature associated with developmental setback in blind and visually impaired children. Although the authors recognized the period around the second year of life as most vulnerable in these children (particularly in those with neurological abnormalities), they pointed out that the individual variability in development and the wide variability in the methodological aspect make it difficult to draw conclusions on the occurrence of developmental setback in blind and visually impaired children.

It is crucial for professionals who are in contact with these children to recognize these developmental risk signs, namely the presence, persistence, and entrenchment of a whole series of behaviors which are expressions of considerable social isolation. These behaviors include remaining in a lying down position, lack of attention towards environmental stimuli, absence of smiling (or problems eliciting smiling), poor adaptive use of the hands to explore and recognize objects, absent or poor "reach on sound" after the fourth trimester of life, and persistence of excessive and non-functional use of the mouth as the main interface with the environment [34].

## 3. Behavioral and Neurocognitive Traits in Visually Impaired Compared to ASD Children

Although visually impaired children do not present a typical personality profile, it is possible to recognize certain frequently occurring traits, namely high levels of anxiety, some difficulties in social interactions, an excessive production of speech (with declarative rather than communicative intent) serving to fill an emotional void, behavioral rigidities [19], that need to be early detected and constantly monitored. There is a remarkable risk that a blind child's personality can be limited to body sensations and that the bridge between the self and the outside world can become unstable or even non-existent. If this issue becomes a source of excessive self-restraint, then the onset of problematic behaviors, such as stereotypes, becomes more common in these children [10,46,47]. A presentation of the most representative behavioral and neurocognitive traits that lead to consider the presence of overlapping symptoms between VI and ASD is listed below.

### 3.1. Joint Attention

Sighted babies and young children use visual behaviors like eye contact, gaze following, and joint attention to set up and sustain communication and to learn about the behavior and intentions of others, especially during the pre-linguistic stage [48]. These early visual behaviors and associated interactions appear to lay the foundation for developing emotionally secure attachments, language, and achieving knowledge about self and others [48]. Joint attention is a triadic relationship that arises in the first months of life, based on mutual gaze between the child, an object, and a social partner, in which both the child and the partner are aware of one another's attention towards an object or event [49]. Visual perception is crucial in this interaction [50].

Joint attention occurs in blind children as well, even if they can acquire it later and differently with respect to sighted children [51]. Infants with VI can be less engaged in joint attention: they usually tend to respond to social interaction with decreased visual attention, pointing [49], or smiling [52]. They are reported to tend to turn head/body away from caregivers and to initiate play interactions with their mothers less often than their sighted peers [13,53]. These behaviors can be interpreted by caregivers as simply a lack of interest, decreasing positive social exchanges [54]. Dale and Salt [48] found that less than a third of the children with profound VI aged 28–40 months were able to share interests and experience with a toy or share interest in an event, in contrast to the great majority (over 80–90%) of the severely visually impaired and sighted children. In a longitudinal study, Urqueta Alfaro and colleagues [54] showed that, in 12-month-old visually impaired infants, the reduction of contrast sensitivity predicted the percentage of time spent in joint engagement. Caregivers of infants/children with VI can learn to interpret and sensibly respond to their baby's signals through non-visual means [55,56]. Rattray and Zeedyk [57] identified touch, vocalizations, and facial orientation as alternative means to maintain the quality of communicative interactions between mothers and their infants with VI, even if it was not explicitly explained. In their study, infants with VI used active touch during shared attention as a tactile form of communication and made use of facial orientation to a lesser degree than touch and vocalizations, indicating that facial orientation is not as important as an alternative communication means [57].

The atypical development of joint attention in infants with VI, compared to their sighted peers' developmental patterns, is considered by some authors as a typical sign of ASD [58]. The emergence of joint attention may in fact be disrupted by ASD [59,60]. However, as recently outlined by Urqueta Alfaro et al. [54], the mechanisms and timelines of joint attention development in infants with VI is obviously different from what is expected in infants with typical development, as described above. Failing to recognize this may put VI children at risk of being wrongly labeled as autistic [54]. However, if in ASD the absence/reduction of interest in shared objects and people is a typical feature, as defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [61], alternative means beyond visual attention is shown in infants with VI to maintain the quality of communicative interactions [62,63].

### 3.2. Language and Communication Skills

Vision is implicated in general language development, as visually driven joint attention experiences in early childhood provide a framework within which language learning occurs [64]. Despite marked variability in visual profiles, children with both peripheral and central VI may exhibit the presence of language and communication disorders [37,64]. This can be a reflection of the visual deficit itself on early interactive experiences, or represent an associated neurodevelopmental condition that occurs independently of the VI or, more frequently a consequence of the two conditions [19].

Communicating with other people can be a challenge both for children with ASD and children with VI, especially as the pragmatic component of language is concerned [64]. As with individuals with ASD [65], children with VI have unique methods of communication (relying instead on non-verbal communication techniques, echolalia, moving from topic to topic, speaking with no eye contact) that may be important in overcoming social barriers.

In children with VI due to central origin, language disorders have been described [37], and may be influenced by both the degree of visual loss and by widespread brain damage that impacts brain network organization and consequently, the development of general neurocognitive functions including language [19].

Language skills have also been widely detailed in children with VI due to peripheral origin and have been considered in the past as the most promising indicator of peripheral VI children's ability to compensate for early deficits in developing inter-subjectivity [66].

Differently from children with ASD, language may be a developmental domain which provides blind children with alternative non-visual strategies for social development [67] but, similar to children with ASD, adverse outcomes in social communication may be also present in children with both peripheral visual impairment (PVI) and cerebral visual impairment (CVI), probably given to disruptions in visually guided experiences and visual behaviors, which are seen as precursor milestones for subsequent social development [62].

Language includes shared understanding of what words mean (lexicon/semantics); the capacity to change words in systematic ways (morphology); and rules that govern word order in a sentence (syntax). Speech and phonology are the oral means of communicating language. The use of language as a social tool (pragmatics) involves a complex set of rules about using eye contact, interpreting nonverbal messages together with words that may have a different literal meaning. In blind children due to peripheral origin, structural language skills, namely phonology, morphology, and syntax, may allow for fluent conversation and have been described as typically developed, differently from most of the autistic children, in which language impairment is reported [61]. On the other hand, semantic and pragmatic skills, that are required for successful socio-communicative functioning, have been described by Tadic and colleagues [64] as being poorer in both VI and ASD.

Mills [68] outlined that children with VI due to peripheral origin usually develop fully intelligible speech within the same time frame as sighted children. In a recent study, Feng et al. [69] showed that they have enhanced attentional sensitivity to "non-visual" components of language such as phonetic-phonological components. Roder and colleague [70] showed that blind participants were more efficient than sighted children in terms of phonological processing. They score consistently higher than their sighted peers on tests of verbal working memory [71–73] as well which, on the contrary, is usually impaired function in children with ASD [74].

With regards to the lexical component, Vinter and colleagues [75] showed that blind children tended to define words denoting concrete animate or inanimate familiar objects evoking their close perceptual experiences of touch, taste, and smell. It was different from what sighted children, who relied their definition on visual perception, and produced more visually oriented verbalism. They also may exhibit atypical conceptual and semantic development [76,77] and demonstrate specific deficits in understanding visual concepts that they have learned through language and not through direct experience. Given fewer opportunities to benefit from traditional classroom education, blind children, due to peripheral disorders, have shown that they may score below their sighted peers on comprehension, similarity, and vocabulary subtest [70,71,78]. Similar to those with ASD [79], young blind children have a limited capacity for generalizing a given word for other items in the category, and use a word for the original referent or only very few items in the category [79].

No significant difficulties with syntactic development have been described in children with PVI [68]. If complexity of structures is analyzed, blind children show similar performance to that of sighted children not only during the first steps of grammatical development, but also taking into account the acquisition of complex sentences [80]. Blind children's morphological development, with the exception of personal and possessive pronouns usage, has not been described as delayed nor impaired in comparison to the one of sighted children [80]. Dunlea and Andersen [81] have suggested that young blind children use few morphemes such as plural, 3rd person of present indicative, and locative prepositions in organizing structures and imitations. Blind children seem to start to productively use pronouns very late (around age 4), and they produce a great proportion of reversal errors (1st person

for 2nd person pronouns and vice versa) [7,81]. On the other hand, language can be delayed in children with CVI [64], whose ability to respond to stimuli has been described by parents as altered [37].

Considering pragmatic aspects, the tendency to use words whose concrete referent is unknown to the speaker, a behavior named verbalism, is another common language behavior of both children with peripheral VI and ASD [82], as is the tendency to use self-oriented language instead of externally oriented language or the tendency to produce a lesser proportion of verbal expressions to offer, show or draw another person's attention [80].

Echolalia represents one of the peculiar ways of communicating found in children with peripheral VI and ASD. However, learning and using whole phrases or formulas for specific contexts and activities allows to participate in social interactions and share activities with other people [83], while the social role of echolalia in ASD is controversial [84]. Like children with ASD, blind children may ask many questions, sometimes inappropriately, and may make 'off-the-wall' comments [83]. They also tend to refer more often to their personal experiences than sighted children when evoking familiar objects [75]. Mothers of children who present severe peripheral VI seem to take more frequent and longer turns at speaking or with other forms of communication than do mothers of sighted children, resulting in an asymmetry between relative dyads' experiences [67,85]. Parents of blind children also tend to use more response control, more test questions instead of real questions, more requests and more repetitions [56], use more imperatives and requests, and were more likely to introduce the topic of conversation [86] than do mothers of sighted children.

### 3.3. Stereotypical Behaviours

The presence of stereotypical behaviors in children with VI has also been observed and extensively reported in several studies [47,87–91]. Although stereotyped movements are a defining characteristic of ASD, there is also some evidence of a distinct pattern in the visually impaired group. Gal and colleagues [91] assessed self-injurious and other stereotyped movements in children with ASD, vision impairment, intellectual disability, or hearing impairment and in typical children. The group with visual impairment had the second greatest prevalence of manneristic behaviors, but it is also engaged in forms of stereotyped movements sufficiently distinctive and rarely present in other groups. Particularly, visual self-stimulatory behaviors, including eye poking, eye pressing, eye rubbing (which may lead to a number of ocular complications including infections, keratoconus, and corneal scarring), light gazing, and staring, form a large portion of the stereotyped exhibited behaviors by visually impaired children [47,88,92–95]. These behaviors are generally exclusive to children with VI and are especially present in children with peripheral visual impairment: Jan and colleagues [96] found that those children with a retinal disorder such as Leber's congenital amaurosis or retinopathy of prematurity were the most intense eye pressers. Other stereotypical behaviors typically observed in visually impaired children are motor stereotypes. These include repetitive head/body rocking, thumb sucking, jumping, swirling, and repetitive hand/finger movements [89,92,93,97–99]. However, in a study by McHugh and Lieberman [94], it has been suggested that body rocking often occurs also in children with retinopathy of prematurity and severe VI. This behavior is most likely to occur in those with a CVI, perhaps because of poor motor development in these subjects [97,100]. Similarly, flickering fingers in front of the eyes while staring at light is common in children with CVI and has been interpreted as an extension of light gazing behaviors [101,102].

Various interpretations of stereotyped behaviors have been reported in the literature [92]. For example, some authors considered eye-digital signs as a means to self-stimulate the sensation of light, producing phosphenes (light sensation that result from mechanical pressure on the eyeball that stimulates photoreceptors and activates intact visual pathways) [47]. Other authors have suggested that these behaviors may be caused by an imbalance of neurotransmitters, especially dopamine and serotonin, due to a damage in the central nervous system [100]. Theoretical approaches have been used to explain stereotypical behaviors from a behaviorist, developmental, and functional perspectives [100]. Specifically, children with VI might acquire and maintain stereotyped behaviors because they are

reinforced by their consequences (e.g., avoiding an unpleasant situation, or drawing attention), because of a delayed motor development (as an expression of neuromuscular maturation processes), or because these behaviors can act as modulators of arousal state, increasing or decreasing the level of stimulation (e.g., thumb sucking in situations of under-stimulation, repetitive hand movements, and jumping in situation of overstimulation) [91,93,100,103]. According to these hypotheses, the frequency of stereotypic behaviors in visually impaired children seems to decrease with age [93,97] and children affected by isolated visual deficits present stereotyped behaviors which are generally more reversible than the ones found in children with additional disabilities [47]. Further, studies have supported the view that the prevalence and the type of stereotyped behaviors are directly related to the severity of visual impairment [91,92,97]. Early intervention is very important in order to stop stereotyped behaviors from becoming established, entrenched, and irreversible [92]. The purpose of this intervention is to provide support, but also to promote opportunities and situations which will allow children to re-establish contact and communication with the world around them. The way VI impacts children's development does not solely depend on the sensory limitation itself, but also on the degree caregivers and society accommodate to these children's needs and strengths [54]. Sensitive parenting in which parents are vocally and tactually responsive to their children's actions facilitates many blind infants' ability to learn their interpersonal effectiveness in the social world.

Instead of focusing mainly on visual attention and facial expressions, parents can be encouraged to become more sensitive to their children's unique inviting signs, pay more attention to the use of movement, touching, tickling, vocalizing, and speech in eliciting physical-tactile and vocal interaction routines [67,104] and to look at body pointing and other unique nonvisual referring signs to create good levels of communication and shared affective meaning about objects and events in the immediate environment [63]. Moreover, the possibility to refer to autobiographical memory is very important in blind children because it is the way they can understand the world. Consequently, unexpected changes in their environment can disturb them and parents should pay attention to guarantee coherence in the personal environment of these children [75].

### *3.4. Theory of Mind*

Baron-Cohen [51] has argued that an individual's eye movements and relationship with a "shared visual attention mechanism" play a key role in establishing a theory of mind module in the developing infant. Hobson [62,105] described foundations of theory of mind and interpersonal understanding in terms of a child taking part in triadic interactions that involve both the child's and the partner's awareness of the other's mutual focus of attention to a third object or event (joint attention). Through joint attention, the child can understand the other person's attitude towards an object [49], and this behavior is usually carried out via the visual modality [105].

Deficits of theory of mind (ToM) in ASD have been related to a lack of inter-subjectivity in ASD children [106]. In other words, an inability to understand and anticipate the thoughts and emotions of others has been associated with a lack of shared social understanding [107]. Children with VI may have difficulties in understanding thoughts and emotions of others as well since, as Bedny et al. [108] highlighted, congenital blindness can alter two important sources of information that can be considered as building blocks of ToM. At first, it does not permit blind children to learn about other people's minds via visual observation of other people's facial expressions or body movements. Secondly, it alters first person experiences of mental life. Specifically, children with VI can understand and share abstract features of other's experience, but could not have the same experience [108]. It is interesting to note that, differently to individuals with ASD, whose ToM disruption is debated since the Baron Cohen's study on 1985 [106], children with congenital VI may present with a delay, but not a deficit in the ToM construction [109], despite not having access to some (visual) information about the mind during development. Eventually, as adults, they can develop a functional and effective ToM, including an understanding of other people's experience of sight [110].

Evaluating ToM in children with VI can be challenging because many tests used rely on visual capacities. This can help explain 4–7 years delay previously described in developing ToM in congenitally blind children [21,111–114]. False belief tasks have been particularly used in the evaluation of ToM in children [109]. The first type of false-belief tasks, in which children are expected to predict or explain another agent's behavior in terms of the agent's mental states (e.g., Baron-Cohen and colleagues' "Sally-Anne" task), have been used in assessing ToM in children with VI [113]. Sometimes, they have been based on tasks in which visual experience has a significant role [112–114]. Because VI can affect the development of ToM, purely due to visual and perceptual deficits, different tasks from the first-order FB have been needed to evaluate ToM in blind children. Second-order FB tasks were introduced later to examine people's belief about others' belief (i.e., "John thinks that Mary thinks that ... " [115]), with positive performance provided by children with VI [109,116]. As a matter of fact, in a recent study, the introduction and use of more reliable tools has identified a similar development of ToM capacities in blind children as compared to sighted peers [109]. In Bartoli et al.'s study [109], 17 children with PVI or blindness underwent an adapted version of the ToM Storybooks and performed similarly to the ones of matched typically developing children, matched on chronological age and gender. Pijnacker et al. [116] administered to blind children several first-order and second-order auditory tasks, showing that the visually impaired children's performances did not differ from sighted children, matched on gender, age, and verbal IQ. These data suggest that the visual nature of the tests or the stimuli should systematically be considered.

Different performance on ToM tasks seem to be related to the type of VI as well. In children with PVI, a delay in ToM development was described in the first studies [21,111–114], not found in the more recent ones [109,116]. Children with CVI may present a more compromised neurocognitive profile than what is usually expected in children with PVI [117]. Begeer et al. [118] found that ToM performances in children whose blindness involved the optic neural pathways were delayed, compared to the performances of children whose blindness did not involve any neural damage. The detected difficulties in interpreting others' intentions and reactions that children with CVI showed, could have reflected the deleterious effect of CVI on the understanding of the social context and facial expressions [37]. These difficulties may also be a consequence of the low IQ levels that children with CVI may present [19] and that are in relation to ToM tasks [111]. As suggested by Bartoli et al. [109], a possible future area of research could compare VI children and children with autism matched on verbal IQ, age, and gender, in order to further understand the role of visual experiences on ToM development.

## 4. Methods Used to Assess ASD in Visually Impaired Children

Since there are no consistent results in terms of the relationship between specific types of ophthalmological problems, severity of VI, and the role of associated handicaps (such as hearing deficits, cerebral palsy, epilepsy, and other intellectual disabilities), and their relationship with ASD, it seems necessary to find a new approach when explaining autistic symptoms in the blind and in the sighted population [18]. ASD is known to be highly heterogeneous, and this has made it hard to define a clear phenotype. Although biologically based and with an evident genetic component [119], ASD is defined and diagnosed based on behavioral difficulties, concerning social interaction and the development of communication skills, and repetitive behaviors and restricted interests. Since ASD is defined by a common set of behaviors, it is best represented as a single diagnostic category that is tailored upon the individual's clinical presentation including clinical characteristics and associated features [120]. Assessing ASD in blind and visually impaired children is a very delicate process in which most of the common methods used to score autistic behavior, including several items linked to vision [121,122] are applied. Therefore, in clinical practice, these standard assessment tools may not be appropriate for specific VI populations [123]. Some authors have designed checklists and/or questionnaires as screening tools to guide further clinical evaluations. Hobson and colleagues [8,20] suggested a checklist containing some clinical features typically found in ASD (derived from DSM-III-R) and used it to interview the children's teachers. Jutley-Neilson and colleagues [32] used the Social Communication

Questionnaire (SCQ), a standardized parent report measure to evaluate communication skills and social functioning in children. Many of the items in the questionnaire involved situations that can only be experienced by sighted children, and the authors highlighted that the SCQ was not as sensitive and specific for visually impaired children. Hoevenaars-van den Boom and colleagues [123] aimed to identify ASD-specific behaviors in deaf-mute people. For this purpose, authors have developed the "observation of characteristics of ASD in persons with deaf-blindness (O-ADB)", an originally semi-standardized observation tool based on the Autism Diagnostic Observation Schedule [124], the Autism Screening Instrument for Educational Planning [35], the Autism Diagnostic Interview Revised [125], and on the Van Dijk Approach to Assessment [121].

The absence of a valid methodology for this population has often led to the conclusion that diagnosing ASD in children with visual impairment should be based on clinical judgment [122]. However, more recent efforts have been made to adjust or modify the assessment tools used to assist with the clinical diagnosis of ASD in VI children. For example, most authors administer the modified CARS and exclude Item VII on visual responsiveness in order to identify children at risk of developing pervasive developmental disorders [8,20,26,28]

Recently, Williams and colleagues [126] have started applying systematic modifications to the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview, Revised (ADI-R) in order to assess symptoms of ASD in visually impaired children (the majority of whom have ONH). This pilot study has provided preliminary evidence regarding how to modify ASD measures which are now more useful in the diagnostic evaluation of visually impaired children and both these tools have shown a good agreement with clinical diagnoses. Authors have concluded that additional research is needed to validate the modified measures in larger samples which may include different diagnoses and levels of visual impairment, and also to follow visually impaired children over time to identify common developmental paths and outline whether specific symptoms change over time [126].

In this direction, a recent study by Fazzi et al. [19] employed systematic modifications (i.e., materials and scoring procedures) to the ADOS 2 [127] (second edition) to assess symptoms of ASD in visually impaired children, taking into account the specificity of type of visual disorder (cerebral vs. peripheral visual impairment). In children with CVI, the use of the modified assessment tool (M-ADOS 2) did not modify the diagnostic category, and the clinical diagnosis matched the ADOS 2 classification and the M-ADOS 2 classification in almost all patients. Conversely, among participants with PVI, 16.9% were classified as autism/autism spectrum in accordance to the ADOS-2 scale but only 10% were confirmed using the M-ADOS 2, exhibiting good concordance with the clinical evaluation result. Although preliminary due to the small sample size, the study suggested that autistic-like finding in children with PVI are more influenced by the degree of VI, and specific symptoms may be more reliable than others in discriminating ASD in VI children. The authors point out the importance of using appropriate adapted tools in PVI subjects to avoid overestimation of ASD that may be confounded by the presence of VI and symptoms and habilitation strategies associated with ASD should take into account possible differences in the context of impaired visual abilities.

The utilization of modified assessment tools, specific not only for ASD but also for VI, matched with a careful clinical observation, is needed in order to ensure a correct diagnoses. As clinicians have independently modified existing autism measures to assess children with VI, future challenges associated with improving the diagnostic precision of ASD in VI will be the development of specific assessment based on visual neutral tasks, detailing modifications so that findings can be replicated, and the validation of these tools on larger sample.

## 5. Conclusions

The relationship between VI and ASD is a controversial issue and it is well expressed by the still controversial estimated prevalence of ASD among visually impaired population.

The current review suggests that some evidences can help us in understanding autistic-like behaviors in VI. ASD among visually impaired children can be a neurodevelopmental condition that

occurs independently of the visual disorder. This seemed to be particularly true for those described subjects who present potential common causal factors, such as genetic defects, prematurity, pathologies that interest the central nervous system. These conditions cause a combination of blindness and brain damage, which is an important contributing factor for the development of ASD.

Autistic-like symptoms can also be secondary to the VI and related to sensory deprivation and environmental risk factors. This is typical of those children who present only severe VI or blindness, without other disorders that involve the central nervous system. In these cases, the underlying pathway of autistic-like features in VI is distinctive of that of individuals with ASD. Peculiar differences can be found, starting from the great interest in shared objects in blind, but not in ASD individuals; good structural language skills that allow for social participation and shared activities in blind, but not in ASD individuals; evidence of potential reversibility of autistic signs as a transient phenomenon in blind but not in ASD individuals.

According to Brambring [128], in these individuals, autistic-like symptoms may reflect blind-specific developmental problems in the acquisition of social-cognitive abilities rather than a psychopathological disorder. In other words, sighted autistic children and blind children may reveal similar symptoms, but for different reasons.

In visually impaired individuals who present associated problems with potential common causal agent, a detailed analysis of autistic-like symptoms is necessary, in order to avoid an overestimation of the co-occurrence of ASD.

Diagnosing ASD in VI children should be done very carefully in clinical practice and assessment tools that take into account the type and level of VI are needed. The future challenge will be to apply new tests involving alternative nonvisual tasks (e.g., based on tactile or auditory experiences) and to improve our understanding of the alternative developmental pathways and adaptive-compensatory approaches in children with VI and autistic-like symptoms.

**Author Contributions:** Conceptualization, A.M., E.L.F.; investigation, A.M., S.M.; writing—original draft preparation, A.M., S.M., A.R., J.G., L.B.M.; writing—review and editing, A.M., S.M., L.B.M., E.M.F.; supervision, F.G., L.B.M., E.M.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Glossary

| | |
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| ADI-R | Autism Diagnostic Interview, Revised |
| ADOS | Autism Diagnostic Observation Schedule |
| ASD | Autism Spectrum Disorder |
| CARS | Childhood Autism Rating Scale |
| CVI | Cerebral/Cortical Visual Impairment |
| DSM-5 | Diagnostic and Statistical Manual of Mental Disorders 5th edition |
| FB | False-Belief task |
| LCA | Leber Congenital Amaurosis |
| O-ADB | Observation of characteristics of Autism in persons with Deaf-Blindness |
| OHN | Optic Nerve Hypoplasia |
| PVI | Peripheral Visual Impairment |
| SCQ | Social Communication Questionnaire |
| SCRR | Social, Communicative, and Repetitive or Restricted behavioral difficulties |
| SOD | Septo-Optic Dysplasia |
| ToM | Theory of Mind |
| VI | Visual Impairment |

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*Review*

# Morphofunctional Alterations of the Hypothalamus and Social Behavior in Autism Spectrum Disorders

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Received: 28 May 2020; Accepted: 3 July 2020; Published: 8 July 2020

**Abstract:** An accumulating body of evidence indicates a tight relationship between the endocrine system and abnormal social behavior. Two evolutionarily conserved hypothalamic peptides, oxytocin and arginine-vasopressin, because of their extensively documented function in supporting and regulating affiliative and socio-emotional responses, have attracted great interest for their critical implications for autism spectrum disorders (ASD). A large number of controlled trials demonstrated that exogenous oxytocin or arginine-vasopressin administration can mitigate social behavior impairment in ASD. Furthermore, there exists long-standing evidence of severe socioemotional dysfunctions after hypothalamic lesions in animals and humans. However, despite the major role of the hypothalamus for the synthesis and release of oxytocin and vasopressin, and the evident hypothalamic implication in affiliative behavior in animals and humans, a rather small number of neuroimaging studies showed an association between this region and socioemotional responses in ASD. This review aims to provide a critical synthesis of evidences linking alterations of the hypothalamus with impaired social cognition and behavior in ASD by integrating results of both anatomical and functional studies in individuals with ASD as well as in healthy carriers of oxytocin receptor (OXTR) genetic risk variant for ASD. Current findings, although limited, indicate that morphofunctional anomalies are implicated in the pathophysiology of ASD and call for further investigations aiming to elucidate anatomical and functional properties of hypothalamic nuclei underlying atypical socioemotional behavior in ASD.

**Keywords:** autism spectrum disorders; hypothalamus; amygdala; oxytocin; social cognition; social interaction; affiliative behavior; neuroimaging

## 1. Introduction

Autism spectrum disorders (ASD) are neurodevelopmental disorders with complex and diversified pathogenesis characterized by dramatic impairment of social communication, social interaction and empathy with an estimated prevalence in the general population ranging from 1 in 100 to 1 in 54 children [1]. ASD are heterogeneous disorders with multisystem and multigenic origin, where even identical genetic variations may lead to divergent phenotypic characteristics [2]. Neuroimaging studies suggested widespread abnormalities involving distributed brain networks [3–7], but convincing evidences of systematic differences in brain network dynamics underlying the cognitive and behavioral symptoms of ASD are still lacking. On the other hand, an accumulating body of evidence indicates a tight relationship between the modulatory functions of the endocrine system and typical and atypical social behavior [8–12]. In particular, two evolutionarily conserved hypothalamic peptides, the oxytocin (OT) and arginine-vasopressin (AVP), because of their extensively documented role in supporting and regulating affiliative and socio-emotional responses [13–17] have attracted great interest for their critical implications in ASD.

Animal studies revealed that OT and AVP critically mediate social and affiliative behavior [18–20]. In addition, administration of OT has been shown to facilitate protective and nursing behavior toward pups [21]. In non-human mammals, OT is generally observed to facilitate approach behavior by decreasing avoidance of proximity and reducing defensive behavior, whereas AVP appears to modulate aggressive responses in relation to pair bonding and mating behavior, especially in males [22,23]. In humans, the effects of intranasal OT administration indicate a reduction of social stress and anxiety facilitating positive social approach and interaction, and affiliative behavior [24–27]. Moreover, intranasal AVP administration in humans, similarly to the effects observed in animals, has been shown to differentially influence social behavior in males and females, with increasing aggressive and agonistic responses in men and facilitation of pair bonding in women [28]. Several investigations also reported an association of the levels of peripheral OT and oxytocin receptor (OXTR) polymorphisms with the diagnosis and severity of ASD [29]. Genomic and epigenetic evidences for OXTR deficiency have been also observed in individuals with ASD [30]. Remarkably, a large number of controlled trials indicated that intranasal OT and AVP administration can ameliorate social abilities in autism [31–36].

Altered OT and AVP synthesis and release appear to be among the core dysfunctions underpinning the impairments in social and communication behavior of individuals with ASD [9,11,37], although it remains unclear whether OT neuropeptide can be used as biochemical marker for ASD [38].

OT and AVP are synthesized by magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus that secret them into the peripheral blood circulation through the posterior pituitary gland. Importantly, these peptides also act as neurotransmitters through the dendritic terminals of magnocellular neurons that release them into the hypothalamic extracellular fluid [39], and through parvocellular neurons projections to brainstem and subcortical regions, such as the amygdala, nucleus accumbens and hippocampus [40,41]. In addition, besides passive diffusion in brain circuits following dendritic release [42,43], OT transmission is also mediated by widespread long-range axonal projections of hypothalamic OT neurons [14] permitting direct modulation of the amygdala and other forebrain regions [20]. Correspondingly, OT and AVP receptors have been localized in various brain regions including the hypothalamus and the limbic system [30,44,45]. Notably, differential OT release mechanisms through dendritic and axonal terminals characterize hypothalamic activity. In fact, dendritic OT release can occur with no spiking activity, and thus, no secretion into the peripheral circulation; vice versa, electrical activity of the cell bodies can induce OT release from axon terminals without central OT release from the dendrites [46,47]. Moreover, dendritic release can lead to a very large disproportion between the concentration of OT in the extracellular fluid of the hypothalamic supraoptic nucleus and that in the periphery by over 100-fold greater [41].

Furthermore, there exists long-standing evidence of severe socioemotional impairment after hypothalamic lesions, involving in particular the ventromedial nuclei [48]. Rage has been observed after ventromedial hypothalamic lesions in both animals and humans (Wheatley 1944 [49]; Reeves & Plum 1969 [50]). Separation-induced distress vocalization can be elicited by electrical stimulation of the medial hypothalamus in guinea pigs (Herman & Panksepp 1981 [51]). Stereotactic stimulation studies in humans showed altered sexual behavior triggered by accidental focal lesions of rostromedial basal forebrain structures including the septo-hypothalamic area [52]. In addition, hypothalamic stimulation can also induce pleasurable experiences and prosocial behavior in humans [53,54]. For instance, several investigations demonstrated reduced aggressive behavior and increased social interactions after deep brain stimulation of the posteromedial hypothalamus [55].

Nonetheless, despite the unquestionable key role of the hypothalamus in the production of the OT and AVP (Swanson and Sawchenko, 1983), the severe socioemotional dysfunctions caused by hypothalamic lesions, and the apparent association between hypothalamic neuropeptides and socio-affective responses in ASD and neurotypical population (NT), hypothalamic involvement remains elusive in most of neuroimaging investigations exploring the neural correlates of normal and abnormal human socioemotional behavior [56–62]. In particular, a surprisingly limited number of studies analyzed the implication of the hypothalamus in the social impairment of individuals with ASD.

Building on the above mentioned evidences, this review aims at providing a synthesis of neuroimaging investigations reporting morphofunctional alterations of the hypothalamus in ASD through the examination of data from individuals with ASD as well as from healthy carriers of genetic risk variation in OT receptors, as several polymorphisms of OT receptor genes have been associated with modulation of socioemotional responses and ASD [63–71]. A description of MR-based anatomical studies reporting abnormal morphology of the hypothalamic region will be followed by a survey of the few existing task-based and resting state functional MRI studies reporting hypothalamic alterations in individuals with ASD and in healthy carriers of genetic risk variation in OT receptors. A critical discussion integrating anatomical and functional findings will then attempt to provide some interpretations of the possible role of the hypothalamus, and its functional exchanges with cortical and subcortical networks, in the atypical socioemotional responses of ASD individuals. In conclusion, some fundamental open questions aiming at elucidating the morphological and functional hypothalamic anomalies and their impact on social cognition and behavior in ASD will be proposed.

## 2. Literature Search

This review is based on a Pubmed and Scopus search aiming to comparatively analyze the current literature until April 2020 using the following keywords "autism" AND "hypothalamus" AND "social." In total, 236 papers were obtained from Scopus, whereas only 22 papers from Pubmed. After refining the search by limiting articles that included the term "MRI," 42 documents remained in Scopus and just one in Pubmed. The remaining publications were then further screened for articles reporting original research studies. Careful inspection of papers, aiming to identify anatomical and functional investigations related to ASD, led to additional rejections of few unrelated papers as well as inclusion of some others missing in the initial literature search, and surprisingly resulted in only 10 relevant scientific publications for our qualitative analysis.

### *2.1. Structural MRI Studies*

One of the first direct evidence linking anatomical alterations of the hypothalamus with ASD was provided by a study assessing structural MRI based measures of brain morphometry in children and adolescents with ASD ($n$ = 52) [72]. ASD individuals with respect to typically developing controls showed significant decrease of gray matter (GM) volume in the hypothalamic region including the supraoptic and paraventricular nuclei, independently of age, IQ or gender. No differences were observed in global volumes of GM, white matter and cerebrospinal fluid.

In another study, hypothalamic atrophy was measured in young male adults with ASD ($n$ = 10) with respect to neurotypical participants using two complementary structural analysis approaches [73]. First, an ROI-based voxel-based morphometry (VBM) analysis applied to the hypothalamic region, delineated through manual segmentation and including voxels in the third ventricular space between the left and right hypothalamus, revealed reduced GM density of the hypothalamus and increased cerebrospinal fluid density in the third ventricle proximal to paraventricular nucleus. Second, an automatic method was applied to a larger cohort of male ASD individuals ($n$ = 41) to estimate ventricular volume of the third ventricle. This method aimed to indirectly validate previous results on the basis of the assumption that relative increase of third ventricle would imply volume reduction of the adjoining tissues. This analysis demonstrated an increase of third ventricle volume that was independent of the lateral ventricles (used as covariate), and thus excluded global brain volume increase.

Recently, decreased volume in the bilateral hypothalamus along with increased volume in the left amygdala and left hippocampus was observed in young children with ASD ($n$ = 14, mean age = 4.5) compared to typically developing children ($n$ = 14, mean age = 4.1) [74]. In addition, the authors observed that the hypothalamic volume was positively correlated with plasma AVP concentration.

In parallel, several indirect evidences of abnormal hypothalamic structure and function in ASD emerged from studies of healthy OXTR risk allele carriers, in particular with the OXTR variant rs53576

that appears to be associated with lowered socioemotional responses [63,75] and is often observed in individuals with ASD [76–80].

One of the first demonstrations in this direction was a multimodal neuroimaging genetics approach that permitted to identify several neural alterations in a large sample ($n$ = 212) of healthy Caucasian OXTR risk allele carriers [64]. Tost et al., using VBM, revealed a significant decrease of hypothalamic GM volume in rs53576 risk allele carriers that correlated with the degree of allele risk. Notably, decreased hypothalamic volume was predictive of a lower prosocial temperament trait in males. Structural correlation analysis, information that has been shown to mirror anatomical connectivity, showed allele-dependent increase of coupling between the hypothalamus and higher-order limbic processing areas, such as the dorsal anterior cingulate cortex, including the paracingulate cortex and amygdala (encompassing high density OT receptors), in rs53576A allele carriers.

In a consecutive study using VBM methods, reduction of GM volume in the dorsal anterior cingulate gyrus and hypothalamus was also associated in carriers of OXTR rs2254298A, another identified genetic risk variant for ASD; this result was mainly related to male carriers [81]. Structural covariance analysis revealed a significant increase in the structural connectivity between hypothalamus and dACG in rs2254298A carriers, similar to that observed in rs53576A carriers. The observed increase of anatomical coupling in healthy carriers of genetic risk variants for ASD may suggest abnormal connectivity related to alterations of several white matter morphological properties as well as atypical functional interactions [82,83].

Additional studies examining brain morphology in individuals with single nucleotide polymorphisms in the OXTR gene related to ASD indicated other alterations of locale brain volumes including the hypothalamus. Inoue et al. [84], adopting a manual tracing methodology for measuring regional brain volume, observed larger bilateral amygdala volume in Japanese adult carriers of OXTR rs2254298A, proportional to the dose of this allele. No significant association of this genotype was instead observed with hypothalamus as well as with global brain volume. In a subsequent analysis on the same data using VBM, stimulated by result of Tost et al. (2011), the same authors reported that rs2254298A was also associated with reduced GM volume in the dACG but not in the hypothalamus and amygdala [85]. However, they observed an interaction effect between gender and rs2254298A genotype in the right hypothalamus, reflecting smaller right hypothalamus volume in females only.

### *2.2. Functional MRI Studies*

Aoki et al. in a focused metanalysis of 13 fMRI studies in ASD individuals during emotional-face processing (considering both emotional-face vs non-emotional-face and emotional-face vs non-face contrasts) observed abnormal functioning of several subcortical regions [86] among which hypothalamic hypoactivity was prominent. In particular, individuals with ASD ($n$ = 226, age ranging from 9 to 37 years) in comparison to NT controls ($n$ = 251, age ranging from 9.2 to 28.6) showed significant hypoactivation of the hypothalamus, and hyperactivation of the bilateral thalamus, bilateral caudate, left cingulate and right precuneus. The comparison of emotional-face to non-face conditions showed a similar activation pattern but hypoactivity was also observed in the parahippocampal gyrus and amygdala, in addition to the hypothalamus. In line with behavioral studies demonstrating impaired emotional-face processing in ASD [56], the observed alteration of subcortical rather than cortical regions during face perception suggested dysfunctional unconscious processes in relation to social cognition. Notably, reduced hypothalamic activity was not observed in each of single studies included in the metanalysis, possibly because of their limited statistical power [87].

Preliminary evidence of a direct association between hypothalamic dysfunction and social interaction was also shown by Chaminade et al. that measured fMRI-based brain responses in ASD individuals ($n$ = 10, mean age 21) during a more realistic and entertaining social behavior consisting of an interactive videogame of the popular "stone-paper-scissors" game [88]. ASD and NT participants played against three different agents: a human being, a humanoid robot endowed with artificial intelligence attempting to win the games by considering previous games' results, and

a computer that randomly generated the three possible responses. A significant interaction effect between Agent (Human, Robot) and Group (ASD, NT) delineated an activation cluster in the left and right hypothalamus, attributed to the paraventricular nucleus, resulting from decreased activity when ASD participants played against the human as compared to the artificial agent, with respect to NT. In addition, functional connectivity analysis of the left hypothalamus revealed a single cluster in the left temporoparietal junction resulting from the interaction effect of Group and Agent. Specifically, a significant negative coupling between the left hypothalamus and left temporoparietal junction (lTPJ) was measured when NT played against the robot and when ASD participants played against the human. Moreover, the coupling observed when ASD participants played against the human, but not against the robot and computer, was negatively correlated with the severity of autistic symptoms measured with Autistic Spectrum Quotient [89]. Interestingly, the decreased modulation of hypothalamic nuclei activity along with negative functional connectivity between hypothalamus and lTPJ, a region associated with anthropomorphization—which is the tendency to attribute human traits to artificial agents—was observed when ASD individuals interacted with a human player, and similarly when NT individuals played against the robot. The anticorrelation between lTPJ and hypothalamus might reflect inhibitory activity exerted by the lTPJ on hypothalamic nuclei that would then result in reduced social motivation and reward for human interactions in ASD.

In line with hypothalamic functional alterations in ASD during processing of emotional expressions [86], reduced hypothalamic activation was also observed in adult carriers of risk OXTR gene mutation for autism [64,81]. Tost et al. (2010), besides abnormal anatomy of the hypothalamus, reported functional alteration of hypothalamic activity during perception of facial expressions (using a Face-Matching Task). In particular, they observed increased fMRI-based connectivity (measured with cross correlation) between hypothalamus and amygdala, and decreased amygdala activation in adult carriers of rs53576A ($n$ = 228) with respect to individuals with the GG genotype [64]. In a subsequent analysis the same authors observed reduced deactivation of the dorsal anterior cingulate and paracingulate cortex associated with healthy carriers of another OXTR gene polymorphism, the rs2254298A [81]. Moreover, differential functional brain connectivity was revealed by genotype-by-sex interaction effect associated with negative coupling of the hypothalamus with dACG and amygdala in male rs2254298A carriers, and positive coupling in females.

Likewise, Wang et al. (2013) demonstrated gender dependent effects of OXTR rs53576 gene variation on hypothalamic functional connectivity in healthy individuals. Specifically, whole brain analyses of local functional connectivity density (FCD) during resting-state fMRI data ($n$ = 270) revealed a main effect of genotype on the local FCD in the hypothalamus and no gender-by-genotype interaction effect, although local FCD in male AA homozygotes was significantly lower than in male G-allele carriers [90]. Additional analysis of gender-by-genotype interaction considering resting-state functional connectivity of the hypothalamic region only showed significantly weaker coupling between the hypothalamic region and the left dorsolateral prefrontal cortex in male AA homozygotes with respect to male G-allele carriers.

## 3. Discussion

Building on the well-recognized role of the hypothalamus in the production of the OT and AVP, and the emerging evidences of an association between activity of hypothalamic neuropeptides and socioaffective responses in ASD and NT population, we here aimed to inspect the current neuroimaging literature in humans in search for evidences of hypothalamic alterations in relation to the core social deficits in ASD. Examination of current structural and functional MRI studies reporting alterations of the hypothalamus in ASD, although rather limited, revealed quite consistent morphofunctional abnormalities. Specifically, two main findings emerged from VBM and fMRI analyses, in both adults and children: anatomical hypothalamic atrophy and functional hypoactivation during face processing and social interaction, respectively.

### *3.1. Hypothalamic Morphological Alterations*

Anatomical hypothalamic atrophy was mainly related to smaller hypothalamic volume in both ASD individuals [72] and healthy carriers of OXTR genetic risk variant for ASD, and to reduced GM density observed in ASD [73]. Notably, in line with gender-dependent differences in the expression of the OXTR gene [91,92], hypothalamic structural abnormalities in healthy carriers of OXTR genetic risk variant for ASD appear not equivalent in males and females and dependent on OXTR variants [64,85].

Sexual dimorphisms of the hypothalamus might follow similar gender-related differences observed in other brain regions including the amygdala, as well as in interhemispheric connectivity, along with differences in hormone-related personal traits, cognition, behavior and psychiatric disorders manifestation [93], ultimately mirroring ASD prevalence that appears larger in males than in females with a male-to-female ratio closer to 3:1 [94].

The observed anatomical abnormality of the hypothalamus is in line with several neuroimaging observations that, although not always congruently, reported morphological changes in ASD in multiple brain regions [95,96], including reduced volume in the social brain network [97–100].

However, it remains difficult to infer the exact neuronal mechanisms leading to hypothalamic atrophy, since variations of multiple properties of GM can equally affect VBM signal. Changes at the level of neuronal cell bodies, glia or neuropils might all contribute to hypothalamic grey matter reduction and differentially affect regulation of central neuropeptides and peripheral hormonal regulation through abnormal synthesis and release. Indeed, postmortem brain analysis in ASD highlighted various anatomical anomalies related to neuronal density and size, dendritic spine density, glia and cerebral vasculature [101]. In particular, lower neuronal density has been measured in human brain regions involved in social behavior such as the fusiform gyrus and amygdala [102–104], as well as in specific layers of ACC [105], possibly reflecting specific hypoactivation of these same regions in ASD.

Moreover, some insights about neural mechanisms underlying hypothalamic atrophy might arise from animal models of ASD. Genetically modified animal models such as the Black and Tan Brachyury (BTBR) mouse model [106,107] and the copy number variants mouse model simulating the 15q11-13 duplication in human (15q dup) [108] were also associated with decreased GM volume of the hypothalamus. In addition, mice carriers of neurexin gene mutations have been associated with fewer oxytocin-expressing neurons in the hypothalamic paraventricular nucleus [109]. Similarly, mice with missense heterozygous mutation in the contactin-associated protein-like 2 (CNTNAP2) were characterized by specific reduction in the number of OT expressing cells in the paraventricular nucleus in association with reduced OT concentrations in brain extracts [110]. In humans, reduced plasma concentration of OT has been indeed measured in ASD [111] and predicted social impairment [29], but no clear evidences of alterations at central level emerged. Some indications suggest a possible correlation between plasma and CNS OT concentrations, but this correspondence seems particularly dependent upon the assessing methods employed [112]. Thus, there are currently no demonstrations of the specific impact of hypothalamic atrophy on OT transmission to brain circuits in humans.

### *3.2. Hypothalamic Functional Alterations*

FMRI studies in ASD revealed hypoactivation of the hypothalamus in relation to face processing, and during interactive play with humans. Likewise, reduced hypothalamic activity during face perception was also observed in healthy carriers of risk genetic mutations for ASD [64].

As for morphometric anomalies, no direct interpretation of the neuronal processes underlying hypothalamic fMRI hypoactivation is yet possible. Decreased BOLD response does not necessarily imply reduced OT/AVP release. Although dendritic and axonal neuropeptides release is generally enhanced by increased action potential frequency, the BOLD signal neither directly nor exclusively reflects neuronal spiking activity but correlates more strongly with local field potentials, which represent postsynaptic activity and integrative soma-dendritic processes [113]. Considering the observed possible uncoupling between hypothalamic spiking activity and dendritic oxytocin release, which can be locally

mediated by intracellular calcium stores independently of action potentials [114], decreased BOLD signal in the hypothalamus might indeed reflect reduced dendritic release of oxytocin.

Animal models of ASD indicate some convergent evidences in relation to the hypothalamic activity. For instance, 15q dup mice showed no hypothalamic activation in response to odor stimulation and resting-state functional hypoconnectivity in a widespread brain network including the hypothalamus [115]. Conversely, hypothalamic activity positively correlated with measures of typical social behavior in rats not responding to exposure to valproic acid in utero, another animal model of ASD [116].

In humans, hypoactivation of the hypothalamus and reduced oxytocin secretion has been observed in eating disorders [117,118]. However, to date, a clear demonstration of any relationship between hypothalamic activity and OT release at central and peripheral level in humans is still lacking. Furthermore, the limited spatial resolution of the considered studies does not permit to correctly attribute hypoactivation to single hypothalamic nuclei, all having diverse functions in the autonomic and central nervous system.

Reduced activation of the hypothalamus was also frequently associated with decreased amygdala activity in both ASD and carriers of OXTR rs53576A allele, in particular during face processing [64,86]. These findings are consistent with several previous studies reporting decreased amygdala activation during face perception in ASD [119,120]; nevertheless, opposite findings were also reported, but they were supposedly ascribable to longer gaze fixation time and higher anxiety level of individuals with ASD [58,121,122].

Hypothalamic nuclei can be controlled directly by the amygdala through the amygdalofugal pathway and the stria terminalis, and indirectly through the bed nucleus of the stria terminalis, which mediates stimulation of the hypothalamic-pituitary-adrenal axis. Projections of the central amygdala to the hypothalamus and brainstem can directly trigger autonomic fear responses [123]. Stimulation of amygdaloid OXT receptors is then assumed to inhibit these efferences' activity so as to decrease aversive responses to socially-relevant stimuli [20,124,125], which would be increased in case of diminished amygdala activation. Accordingly, increased hypothalamic activity and amygdala deactivation appear to mediate initiation and consolidation of social relationship in healthy individuals. Such a reverse activation pattern of the hypothalamus and amygdala has been associated with several social behaviors such as other people's trust and trustworthiness [126] and mother–infant and pair bonding [127–130], as well as visual processing of personally known faces including same-sex sibling and best friend with respect to unknown faces [131].

Prosocial behavior can be enhanced by hypothalamic through the modulation of two complementary responses: enhancement of social stimuli saliency processing and reduction of fear and avoidance behavior, both mechanisms being strictly dependent on amygdala activity [132,133]. Notably, AVP and OT have opposite modulatory effects on fear and anxiety-related behavior: the former by enhancing sympathetic responses such as stress level, anxiety, aggressiveness and boosting fear memory consolidation, the latter by acting on complementary parasympathetic responses that facilitate prosocial attitude and interactions as well as extinction of conditioned avoidance responses. These opposite regulatory neurophysiological processes result from activation of distinct elements of an inhibitory network within the medial part of the amygdala, and consecutive integration of different afferences to the central amygdala into a modulatory output to the hypothalamus and brainstem for appropriate anxiety and fear responses [134].

In addition, the hypothalamus can significantly influence socioemotional responses through a complex network that includes widely distributed, mostly bi-directional, neural connections to other brain regions. The hypothalamus is interconnected with basal forebrain areas such as the periamygdaloid region and the septal nuclei and other brainstem nuclei through the medial forebrain bundle, which mediates top-down modulation of both somatic and visceral activity by the forebrain and limbic system, as well as bottom-up influences of higher brain activity by internal organs and bodily interoceptive signals.

Previous studies reporting hypothalamic activation concurrent to other socioemotional-related brain regions indeed indicated widespread interactions of the hypothalamus with emotional, motivational and social brain centers [132,135–138]. However, the still scarce evidence of functional connectivity of the hypothalamus in both NT and ASD individuals, which might also be partly dependent of the variable association between hypothalamic spiking activity and oxytocin release, do not permit to clarify how this region interacts during typical and atypical socioemotional behavior.

Indirect indications about alterations of functional connectivity emerge from studies on healthy carriers of risk genetic mutations for ASD. Tost et al. (2010) measured increased structural and functional connectivity during face perception between hypothalamus and amygdala in OXTR risk allele carriers, suggesting a dysfunctional coupling underlying inappropriate responses to socially-relevant stimuli, although the actual nature of their interactions remains unknown. In addition, the same authors observed a negative coupling between hypothalamus and dorsal anterior cingulate and paracingulate cortex resulting from respectively decreased and increased activity [64]. Direct projections of the anterior cingulate cortex (ACC) to the hypothalamus have been demonstrated in both animals and humans [139,140]. Interestingly, concurrent increased activity in the paracingulate cortex and in the septal area, including the hypothalamus, has been associated with unconditional trust towards other people [126]. Maladaptive changes in trusting behavior, for instance after repeated violations of trust, consequent to exogenous administration of oxytocin, have been associated with increased ACC activity and decreased amygdala and midbrain activation [141]. The anticorrelation between the hypothalamus and ACC might then result from exaggerated ACC inhibitory activity of the hypothalamic nuclei preventing adaptive social behavior. The ACC is an important regulatory center that, through direct projections to the amygdala, insula, ventral striatum, hypothalamus and brainstem [140] can control socioemotional responses. Remarkably, it has been proposed that the medial prefrontal cortex, including ACC, would encode abstract representation of social experiences [142] permitting to predict and guide social goal-directed behavior based on social prediction error [143,144]. In line with this assumption, ACC connections with brain regions related to emotion and reward such as OFC, ventral and dorsal striatum, amygdala, insula and hypothalamus would then support generation of active inferences of affective, interoceptive and reward values [145,146] of socioemotional responses, as well as the minimization of prediction error between expected and actual behavioral outcome, the latter seemingly compromised in ASD [9,147].

### *3.3. Relevance of Hypothalamic Alterations in Healthy Carriers of Genetic Risk Variation in OT Receptors*

Structural and functional alterations of the hypothalamus in individuals with polymorphisms of the OXTR gene are intriguing considering the increasing evidence indicating their relationship with ASD [79]. For instance, the OXTR rs53576A and recently the rs2268498 were associated with ASDs in both Asian and Caucasian populations [76,77,148,149]. Despite some inconsistency of the studies linking OXTR rs53576 variant with impaired socioemotional traits and behavior [150], the rs53576 and rs2254298 OXTR single nucleotide polymorphisms were shown to correlate with increased severity of social deficits in ASD, and less with social deficit in ADHD, thus indicating a differential relationship between this neuropeptide receptor gene allele and the social phenotype [80]. A metanalysis on the relationship between the OXTR rs53576 variant and human sociality indicated a clear influence of this OXTR polymorphism on individual psychological traits related to social responses to other people (for instance extraversion, empathy, and social loneliness) [151]. In short, neuroimaging findings in healthy carriers of OXTR rs53576A and OXTR rs2254298A genotypes indicate that alterations in the expression, and possibly function, of OXTR gene might be related to abnormal morphofunctional characteristics of the hypothalamus in ASD. However, it is conceivable that other OT signaling genes, such as the structural gene for OT (OT/neurophysin-I) [152] and gene for OT secretion (CD38) [153] that along with the OXTR have been frequently linked to human social behavior [154], might also contribute to structural and functional brain alterations in ASD.

## 4. Conclusions

The few studies that have thus far observed, directly or indirectly, a relationship between the hypothalamus and ASD indicate both structural and functional alterations. However, considering the paucity of current investigations, further well-defined studies are strongly needed to clarify morphological and functional properties of hypothalamic nuclei and their complex functional exchanges with cortical and subcortical networks during socioemotional behavior in ASD (Figure 1).

- What are the specific phenotypic expressions of morphofunctional alterations of the hypothalamus?
- Do morphofunctional hypothalamic alterations have similar phenotypic expressions in neurotypical and ASD individuals?
- How structural and functional characteristics of the hypothalamus manifest across development in ASD?
- Can morphofunctional hypothalamic alterations help to characterize subtypes of ASD?
- What is the contribution of structural gene for oxytocin and gene for oxytocin secretion in the hypothalamic alterations?
- What is the specific role of anatomical and functional subdivisions of the hypothalamus in atypical socioemotional behavior?
- What is the dynamic functional connectivity between the hypothalamus and amygdala in ASD?
- Does task-related modulation of hypothalamic activity in ASD reflect dynamic changes of oxytocin concentration at peripheral and central level?

**Figure 1.** Questions for future research.

Current neurophysiological investigations on the role of the hypothalamus in typical and atypical human social behavior have been likely hindered by several limitations related to the experimental methodology and MR signal acquisition techniques, resulting in a surprising disregard of its essential contribution. Designing protocols that permit to assess neural activity during realistic and entraining social scenarios, with rigorous control of experimental variables, for both NT and ASD individuals, is particularly challenging. In addition, MRI acquisition schemes adopted in most of previous anatomical and functional brain investigations in ASD were not specifically tailored for the hypothalamus. Neuroimaging of the small hypothalamic nuclei is certainly arduous as needs very high spatial resolution to clearly delineate their functional subdivisions and at the same time it requires prevention of potential partial volume effects, compensation for signal-dropouts occurring in ventromedial subcortical regions and correction for distortions generated by neighboring ventricles and blood vessels. Nevertheless, extraordinary progresses in high-field and ultra-high-field MRI techniques indicate feasibility of high-resolution structural [155,156] and functional [157,158] imaging of the human hypothalamus, and might then valuably support the elucidation of morphological and functional properties of this region in typical and atypical socioemotional behavior. Ultimately, greater understanding of the human hypothalamic morphology and functions is essential not only for the comprehension of socioemotional behavior but also in relation to the direct implication of hypothalamic neuropeptides in synaptic activity and plasticity [37], and neurogenesis [159], that may considerably impact the still obscure pathophysiology of ASD.

**Author Contributions:** A.C. conceived the study, reviewed the literature and wrote the paper; L.C. reviewed the literature and wrote the paper; S.d.F. wrote the paper. All authors have read and agreed to the published version of the manuscript

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Review*

# Theory of Mind Deficits and Neurophysiological Operations in Autism Spectrum Disorders: A Review

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Received: 1 June 2020; Accepted: 18 June 2020; Published: 20 June 2020

**Abstract:** Theory of Mind (ToM) is a multifaceted skill set which encompasses a variety of cognitive and neurobiological aspects. ToM deficits have long been regarded as one of the most disabling features in individuals with Autism Spectrum Disorder. One of the theories that attempts to account for these impairments is that of "broken mirror neurons". The aim of this review is to present the most recent available studies with respect to the connection between the function of mirror neurons in individuals with ASD and ToM-reflecting sensorimotor, social and attentional stimuli. The majority of these studies approach the theory of broken mirror neurons critically. Only studies from the last 15 years have been taken into consideration. Findings from electroencephalography (EEG) studies so far indicate that further research is necessary to shed more light on the mechanisms underlying the connection(s) between ToM and neurophysiological operations.

**Keywords:** EEG; autism; theory of mind; adults and adolescents

## 1. Introduction

Autism Spectrum Disorder (ASD) is a complex pervasive neurodevelopmental disorder, presenting great heterogeneity with respect to symptomatology and traits. With regards to the degree of severity, ASD reveals impairments in many domains such as social interaction, verbal and non-verbal communication, and restricted and repetitive behaviours [1]. Regarding cognitive and social abilities, it has been shown that individuals with ASD [2] present great variability. The spectrum roughly ranges from high-functioning autism to low-functioning autism associated with learning impairments and disabilities [3].

In most of the cases, ASD is connected with mental disability, difficulties in movement coordination, attention deficits, sleep disturbances and gastrointestinal disorders [4]. However, it is not uncommon for some individuals within the spectrum to achieve high performance skills in visual abilities, music, art and mathematics [5]. Research so far has shown that the appearance of the disorder is estimated at 1–2%, is 4.5 times more frequent in males than in females and could emerge in all national and socio-economic strata [6]. It should be noted that impairment in social skills is one of the fundamental characteristics of the disorder, accompanying the individual throughout his or her lifespan [7].

As advancements in cognitive neurosciences have drawn attention to neurobiological features of ASD, there is a great need to understand the disorder mechanisms. Various theories have been proposed; however, the most prominent to consider for the majority of the social dysfunction traits has been Theory of Mind (ToM), which relates to the ability of individuals to evaluate the behaviour of others on the basis of their own mental states, such as goals, feelings and beliefs [8] and enables the identification of others' intentions, emotions, as well as self-awareness [9].

## 2. Theory of Mind

ToM is the ability to interpret the mental states of oneself and others [10] and allows individuals to make considerations as well as reasonable explanations regarding the behavioural patterns of others [11]. However, in the case of individuals with ASD, asymmetry between their own knowledge and that of others is often detected [12]. This is why poor performance in ToM tasks is observed in individuals with ASD [13].

Although ToM is unfounded as an exclusive explanation for the characteristics of ASD, the influence of ToM on social skills is paramount [14]. Individuals with ASD show impairments in the reasoning of intentions and emotions that highlight social conventions [15]. The performance of children with ASD in advanced ToM tasks correlates with their social competence; however, the practice of ToM skills in everyday life is often diminished [16]. It is therefore evident that, in spite of the ability of some children with ASD to generate thoughts, beliefs and intentions in ToM tasks, they are unable to implement these skills in social situations [17,18].

Impairments in ToM abilities in children with ASD lead to social, behavioural and communication deficits, as well as discrepancies in social interaction, due to the inability of individuals with ASD to perceive that behaviour is driven by mental states [19,20]. Social dysfunction can be therefore attributed to the delayed or incomplete acquisition of ToM in ASD; however, individuals within the spectrum show individual differences with regards to the acquisition of those skills. In fact, children with ASD who succeed in ToM tasks are considered to be better socially integrated compared to their ASD peers who fail in those tasks [21,22].

Furthermore, factors in ASD such as social and communication experiences, interaction with parents, inability to process information, weak central coherence and lack of motivation, as well as perception problems prohibit the development of ToM [15,22]. Spontaneity in relation to ToM stimuli and reciprocal socio-psychological cues is totally absent in individuals with ASD, even in the case of high-functioning autism. That being said, individuals with ASD exhibit significant deficits in the process of basic emotion recognition judged from information acquired just from the eye gaze of other people. High-functioning individuals within the spectrum are, however, able to interpret mental states based on the whole facial expression [23]. In all cases of ASD adults, though, there is a lack of spontaneous capacity to attribute mental states.

## 3. Mirror Neurons–Mu Suppression

### *3.1. Mirror Neurons and ToM*

The "broken mirror neurons" theory has received attention in literature with respect to possible connections between autistic traits and discrepancies in the function of mirror neurons (MN); it is hypothesised to constitute one of the factors responsible for ToM attenuation in individuals with autistic traits, and is linked to the interpretative neurocognitive theory of social and communication impairments in ASD [24].

MN delineate a functional set of neurons located in the cerebral cortex, activated both during the performance of an action, as well as during the observation of the performed action [25]. They were designated as such due to their ability of mirroring behavioural patterns, enabling the observers to encode the intentions behind the observed action sequences and to be in a position to further imitate them [26]. This set of neurons is mainly found in the inferior frontal cortex, the premotor cortex, the supplementary motor area, the primary somatosensory cortex and the inferior parietal cortex [27] and is hypothesised to be directly related to social abilities and skills in primates and humans, including imitation, empathy, ToM and language development [28–30]. Due to the fact that individuals with ASD demonstrate impairments in all the aforementioned domains, it is suggested that the system of MN is dysfunctional in ASD [31,32].

The mechanism underlying the activation of MN is strongly linked to imitation ability and imitation-based learning [33], more precisely the imitation of gestural movements and facial

expressions [34]. The inferior frontal cortex and the ventral premotor cortex play a compelling role in the action of facial imitation and mimicry, which is essential for empathy to emerge on a neurobiological level [34,35] and mirrors the synchronous coupling of behavioural and emotional development through non-verbal communication [36]. The inferior frontal cortex has a specific significance in the process of defining intentions or goals by delegating those intentions to representations of internal states, as well as to the transmission and perception of emotional states that are connected to the imitation of facial expressions [37].

Imitation processes depend on the perception of action of the sensorimotor system. The prerequisite of these processes is the elicitation of imitation through external motor stimuli that are identified and executed as action that initiates imitation as response to those stimuli [38]. One of the theories that attempt to provide an explanation for the initiation of imitation is the ideomotor theory of action, which suggests that it is not the motor property of action that triggers a reaction, but rather the goal and intention that defines this action. Iacoboni (2009) mentions that "the coactivation of the intended goal and the motor plan required to achieve it—according to the ideomotor framework—is the result of our experience. We have learned the effects of our own actions, and we expect certain effects when we perform certain acts. This previous learning makes it possible that just thinking about the intended goal automatically activates the representation of the action necessary to obtain it." [39] (p. 655).

MN are theorised to be in the centre of the process of perceiving the intentions behind an act, which further facilitates the emergence and establishment of empathy [40], and plays a significant role for the foundation of common objectives and motives among individuals [41]. Dysfunction in the system of MN in ASD has an impact on the comprehension of action and intention. In particular, individuals with ASD present deficits in perceiving the motor action and the reasoning of an action [42]. It is hypothesised that MN are the substratum of human cognition and social understanding and that their operation facilitates the process of accessing and perceiving the emotional state of others as the result of one being able to reflect one's own individual internal states and experiences [41,43–46].

### *3.2. Mu Suppression in Literature*

A method to investigate the activation of MN in humans is through measuring *mu* suppression. *Mu* is a type of rhythm that can be described as the frequency band 8–13 Hz and can be detected in an EEG test. The modulations of the power of the *mu* frequency band can provide evidence for the specific functionality of the MN in terms of the comparison between an active condition and a baseline condition [47,48]. It is still under question whether the suppression of the *mu* rhythm is a sufficient method for measuring the operational activation of the Mirror Neuron System (MNS), mostly due to small sample sizes in the research studies (especially when examining atypical populations, such as ASD) or the fact that it is mainly the power modulations of the central electrodes that are taken into consideration [49].

Despite the fact that the theory of broken MN in individuals with ASD has attracted attention, it has also created a debate with regards to its plausibility and application. The hypothesis of dysfunctions in MN accounts for deficits in ToM and imitation, but nevertheless, literature has critically approached the theory, suggesting that the aberrant operation of the system of MN does not provide efficient reasoning for the aforementioned deficits, but that it is sensorimotor impairments in ASD that have an impact on the interpretation of actions. This theory derives from the observation of animal behavioural patterns that indicate the ability of comprehension of action without being in a position to reproduce or imitate it [50]. As a consequence, only a small body of literature has investigated abnormalities in mirror neuron functioning in individuals with ASD, especially in the cases of adolescents and adults. The majority of the studies using brain activity screening techniques focus on the activation of MN in very young populations (children or infants), and their findings demonstrate impairments in the function of these neurons during ToM and imitation tasks [51,52] or gestural movements [53,54]. Although there is an adequate body of literature investigating the involvement of MS in the performance and observation

of mentalising tasks in neurotypical adults [55,56], the studies observing this performance in adult individuals with ASD are limited.

Cole et al. [57] examined the activation of MN in young adults with ASD during intention mentalising tasks, in order to detect a possible link between aberrant mirror neuron activity and autistic traits. They recruited 43 participants that matched in terms of age and verbal IQ level, dividing them into three groups according to their level of autistic traits as evaluated by the Autistic Spectrum Quotient (AQ; Baron-Cohen et al., 2001): low AQ (n = 15, mean age = 23.40), high AQ (n = 15, mean age = 24.13) and ASD (n = 13, mean age = 28.30). The participants were required to watch short videos demonstrating motor actions performed by actors that were divided into two categories: a mentalising task and a non-mentalising task. After the end of the videos, they had the task of deciding upon either the intention of the performed action (mentalising) or its success (non-mentalising). The data derived from an EEG screening test performed during the viewing of the tasks, in combination with an eye-tracking test and a Transcranial Magnetic Simulation (TMS)-induced electromyography (EMG). Their EEG findings demonstrated a lower level of *mu* suppression in the right hemisphere in participants within the group with high autistic traits during the mentalising task, which did not, however, correlate with the quality in mentalising performance. On the other hand, a lower performance in the mentalising task positively correlated with a poorer activation of MN in the left hemisphere; nevertheless, this was not linked to the level of autistic traits of the participants. Data derived from TMS revealed no variation between the groups in terms of the activation of MN and its link to performance in mentalising tasks. The hierarchical categorisation of autistic traits was a predictive factor for *mu* suppression in the 8–10 Hz band for the mentalising task and therefore for poorer mirror neuron firing in the right hemisphere. During the non-mentalising task, however, no low level *mu* suppression was detected. The authors attribute the poorer activation of MN in the right hemisphere in individuals with ASD to an abnormal connectivity among MN and the process of mentalising intentions deriving from actions, which is in accordance with the theory of impaired ToM in ASD.

The observation or mentalising of movement, as well as the imitation or execution of the movement, has been associated with the suppression of the *mu* rhythm [58] and has attracted the interest of research, so as to disentangle the relations that underlie the deficits in imitation and reduced *mu* suppression in ASD. In an earlier study, Bernier et al. [59] conducted a study aiming to investigate this link, hypothesising that individuals with ASD will present an impaired imitation ability in correlation with a low suppression of the *mu* wave. They conducted an EEG imitation experiment examining 14 male adults diagnosed with ASD and 15 neurotypical controls, matched in age (18–44 years), gender and intelligence quotient. The experiment included four condition states: (a) resting state, where the participants were required to just sit still, positioning their hands on their lap, (b) observation state, where the participants had to observe a person grasping a manipulandum, (c) execution state, where the participants were instructed to grasp the manipulandum in the exact same way they saw the person doing, and (d) imitation state, where the participants were required to imitate the instructor grasping the manipulandum (experiment adapted from Muthukumaraswamy et al. [60]). The findings with regards to the resting and execution state conditions did not show differences in *mu* suppression between the two groups (reduced in the resting state and increased in the execution state). Nevertheless, the ASD group demonstrated a significantly reduced *mu* suppression in the observation state condition in contrast to the neurotypical controls, which further supported the hypothesis of an impaired execution/observation system in ASD and therefore identified deficits in imitation abilities. The authors, however, observed that this system could not be totally impaired, since individuals with ASD do not entirely lack the ability to imitate but rather demonstrate poor imitation performance. These findings are in alignment with discrepancies in ToM abilities and attenuations in social integration. The study concluded that the execution and imitation of human movement was connected with impairments in *mu* suppression in ASD, further implying a possible involvement of a dysfunctional MS.

Fan et al.'s [61] study dealt with the index of *mu* suppression in relation to the observation/imitation mechanisms, with the intention to challenge the theory of "broken mirror neurons" in ASD. They conducted an EEG study focusing on *mu* suppression as a factor to measure resonance in the sensorimotor system during the observation and imitation of gestural movements. The researchers recruited 20 male adolescents and young adults with ASD (11–26 years) and 20 neurotypical individuals matching the ASD group in terms of age and intellectual abilities. The experiment included an eye-tracking recording and an EEG recording during the conducting of a test containing four conditions: *baseline* condition (observation of a static object on a screen), *hand* condition (observation of a video-recorded gesturing action), *dot* condition (observation of a video with a white dot), *execution* condition (manipulation of an object in the same manner as in the *hand* condition). Their findings constitute strong evidence against the theory of broken mirror neurons in ASD. More in particular, the *mu* suppression occurring from the EEG monitoring under the conditions of observation and imitation of a gestural action did not show significant variation among the two groups. Predominantly, the results did not reveal any correlation between imitation performance and *mu* suppression, contradicting the most up-to-date research findings [32]. The activation of MN in the ASD participants was evaluated as being preserved, and the *mu* rhythm was very close to that of neurotypical individuals, despite the fact that the performance in the imitation condition was significantly lower in the ASD group. The findings also reveal a relation between attenuated communication capacities and a weak *mu* rhythm, indicating a variation in the symptomatology of ASD. Age progression was not found to influence the results in either the ASD or the control group.

When dealing with impaired ToM, empathy is one of the most prominent attenuated social cues, and it is a hallmark of ToM deficits and social discrepancies in ASD. The study of Fan et al. [62] aimed at investigating the empathic and social understanding abilities of neurotypical individuals and individuals with ASD, in order to disentangle the different factors that contribute to the variances with regards to empathic arousal and the perception of social cues in ASD. Their participants consisted of 24 ASD and 21 controls who participated in an fMRI experiment evaluating pain empathy, and 20 adolescents and young adults (16–29 years of age) and 20 age-matched neurotypical controls who participated in an EEG/ERP experiment. A set of 48 images illustrating injured and non-injured body parts were presented, distinguishing between intentional and unintentional injuries as well as individual pain vs. dyad pain situations; these had to be evaluated by the participants with respect to the level of pain. The results of the study demonstrated lower pain thresholds detected in individuals with ASD in comparison to their neurotypical peers, who presented increased hemodynamic responses in SI/SII, stronger N2 but weak responses in the anterior mid-cingulate and anterior insula, and preserved LPP in the view of unintentional body harm, whereas in the case of intentional injuring, they presented reduced LPP and decreased hemodynamic responses in the medial prefrontal cortex. *Mu* suppression and MN activation in view of injuries appeared to be preserved in ASD individuals, similar to the control group, which in combination with an elevated hemodynamic response in the area of the amygdala and higher PPT indicated that individuals with ASD evaluated the pain of others lower due to an impaired perception of social cues. Their emphatic engagement, however, appears to be high, which contradicts the hypothesis of discrepancies in empathy in individuals with ASD.

Another study that examined the hypothesis of attenuated MN activation and *mu* suppression in adults with ASD in terms of decoding the intentions deriving from motion observations and execution is that of Dumas et al. [49]. The aim of the study was to investigate the validity of this hypothesis for the totality of the brain, focusing on two sub-bands of alpha-*mu* bands (8–10 Hz and 10–12/13 Hz), in contrast to other studies that accept a homogeneity of *mu* suppression in terms of frequency (8–13 Hz) and take into account only the electrodes C3/C4, which are located in the centre of the scalp. They examined ten high-functioning adults with ASD and thirty neurotypical individuals matched in terms of age (20–39 years of age) in a three-condition experiment: simple observation of gestures, free imitation of gestures and imitation of a pre-recorded video. Their findings revealed variations in the *mu* response once two bandwidths of alpha-*mu* were considered. More particularly,

the differentiation was detected in the upper sub-band of the sensorimotor region in the ASD group under the condition of a gestural observation, whereas the two groups did not show significant variations in the response of the lower sub-band. The increase in the lower *mu* rhythm band was found atypical, whereas in the higher alpha frequency band it appeared to be normal for the observation condition. On the other hand, the responses to the condition of execution were found normal. The study questions the hypothesis of global impairments in the function of MN in ASD, dissociating attenuations in the process of intention perception from them.

As mentioned above, MN are hypothesised to fire during the observation of an act and could possibly be involved in facial-recognition processing as well as mimicry and imitation processes, reflecting emotional states and ToM abilities [63]. Deschrijver et al. [64] questioned the efficacy of dysfunctional MN as the reason behind deficits in motion observation and imitation abilities in individuals with ASD. Their study aimed to shed light on the cognitive processes that underlie imitation control and imitation impairments in ASD, giving emphasis on three EPR components, the P3, the N190 and the RP in terms of congruency in the stimulus conditions. They tested 23 adults diagnosed with High-Functioning Autism and 23 neurotypical controls matched in terms of age (22–46), handedness and gender. The participants were part of an EEG experiment, during which they were required to observe a videotaped hand performing gestures and to execute a pre-instructed hand gesture right after, under three different conditions: a congruent condition, when the action they were required to execute matched the gesture shown in the video, an incongruent condition, when the gesture they were required to execute did not match the gesture shown in the video and a baseline condition, when the hand shown in the video was in a resting state. The study was the first to conduct a neuroimaging experiment examining the imitation–inhibition task. The findings with respect to the P3 ERP component did not confirm the original hypothesis, demonstrating no significant variation between the ASD group and the neurotypical controls. In that respect, both the individuals with ASD and neurotypical participants generated larger numbers of P3 component during the observation of the congruent gestural action with the gesture they intended to perform. The ASD participants showed the ability to distinguish between compatible and incompatible observed gestures to the intended hand gestures when the processing level was higher. With respect to the RP (readiness potential) Laplacian, the findings suggested that the ASD group had a larger number of RP components for the congruent trials than for the baseline trials. The same effect was also observed for the incongruent trials, which elicited larger P3 Laplacian than the baseline trials. That finding suggests that, in individuals with High-Functioning Autism, the cerebral work load in terms of motor preparation is equally high both when observing a compatible or an incompatible gesture to the planned hand gesture, whereas the influence of the baseline condition appears to be neutral. Unexpectedly, no significant variation was indicated among the two groups in terms of compatible and incompatible conditions. The effect of the intended action on the processing of early visual stimuli (N190), as found in previous studies, could not be replicated in this study. The results postulate the theory that automatic imitation does not exclusively depend on the disentangling of socio-cognitive cues but rather on motor preparation, contrasting the hypothesis of dysfunctions in MN in ASD.

The overall results of studies investigating the relationship between *mu* suppression in ASD and dysfunctional MN are far from conclusive. Aberrant *mu* suppression was not found to be systematically associated with dysfunctional MN, which casts doubts on the robustness of *mu* suppression as a reliable proxy for the functioning of MS or/and the appropriateness of the methodological techniques that have been employed so far in relevant research. This calls for a more in-depth examination of the function of MN and impairments of individuals with ASD at the neurobiological level, as well as interventional methods without invasive techniques. An interventional approach that has received attention in research is the Transcranial direct current stimulation (tDCS), which is a non-invasive cerebral stimulation technique that modulates cortical excitability by applying a low direct current through a set of electrodes on the scalp. In recent years, research using tDCS has gained ground as a great opportunity to causally test the role of specific neural circuits in certain motor or cognitive

functions [65]. Enhanced cortical excitability is found to be linked to anodal stimulation, whereas a weaker excitability is associated with cathodal stimulation [66]. The technique has already found application in measuring modulations in attenuated mirror neurons aiming at a potential decrease of the clinical manifestations of individuals with ASD [67], and also as a treatment method for other clinical conditions accompanied with cognitive impairments, such as schizophrenia or Alzheimer's disease [68,69].

Another important point to consider is the connection between ToM, joint attention and brain connectivity. Joint attention in particular is considered to be a predictive marker for ToM, relying on the efficient integration of information regarding mental states of oneself and of others. It therefore requires successful cooperation among the activation of perceptual neural networks. Deficits in joint attention abilities result in impairments in social engagement, constrain shared intent and imitation, and further diminish the chance of social integration and shared experience opportunities [70]. Jaime et al. [71] examined the EEG coherence during the state of perception of compatible and incompatible joint attention as well as an eye-open resting state. The researchers tested 16 high functioning adolescents with ASD (mean age: 16.2 years) and 17 neurotypically developing controls (mean age: 16.5 years). The participants were presented with 12 short video clips containing a moving red dot and a human model, evoking joint attention with two conditions: a congruent one, where the human model was following the dot with their gaze, and an incongruent one, where the human model was not. The findings of the study showed a low alpha coherence in the central-temporal area of the right hemisphere in the ASD group, which is in alignment with the findings of research studies investigating EEG coherence in adults and children [72,73]. The condition of congruence in joint attention perception did not act as an influencing factor for EEG coherence, neither in the ASD group, nor in the control group. The authors interpreted this finding as supporting that adolescents with ASD have no dysfunction in the frontal-parietal attention-oriented network. Overall, the results support the theory of underconnectivity in ASD. The theory of underconnectivity offers a different dimension, postulating that an aberrant frontal-posterior interaction exacerbates the communication and information exchange between the frontal and the posterior regions that are involved in cognitive activities such as joint attention.

Table 1 below provides an overview of the studies that were selected to be reviewed in this paper:.

**Table 1.** Overview of the selected research studies for this review.

| Authors | Participants | Method | Findings |
|---|---|---|---|
| [57] | 43<br>(mean age ~25)<br>with autistic traits | EEG,<br>Eye-Tracker, TMS-EMG | Lower level of *mu* suppression in the right hemisphere in ASD during mentalising task. Positive correlation of lower performance in mentalising task with poorer activation of mirror neurons in left hemisphere, but not linked to the level of autistic traits.<br>Autistic traits predictive factor for *mu* suppression in the 8–10 Hz for mentalising task and poorer mirror neuron firing in right hemisphere. During non-mentalising task, no low-level *mu* suppression detected. |
| [59] | 29<br>(14 ASD and 15 controls)<br>Age 18–44 | EEG | Poorer imitation ability in ASD.<br>Significant *mu* suppression in the execution of an action among both groups. In the action observation condition the ASD group showed a reduced *mu* suppression. |
| [61] | 40<br>(20 male ASD<br>and 20 controls)<br>age 11–26 | EEG | No significant variation among groups in *mu* suppression occurring from EEG monitoring of observation and imitation of a gestural action. Stronger *mu* suppression during gestural action observation than dot observation in ASD.<br>No imitation of the observed action while MNS activation intact in ASD.<br>Relation between attenuated communication capacities and reduced *mu* rhythm. |

**Table 1.** *Cont.*

| Authors | Participants | Method | Findings |
|---|---|---|---|
| [62] | 40 (20 ASD and 20 controls) age 16–29 | EEG/ERP, fMRI | Reduced pain thresholds in ASD. Heightened empathic arousal. Attenuated social perception in the view of the pain of others. |
| [49] | 40 (10 ASD and 30 controls) age 20–39 | EEG | When *mu* frequency distinguished into two sub-bands, a differentiation observed in the upper sub-band (10–12/13 Hz) of the sensorimotor cortex in ASD in the condition of gestural observation; no significant variation in lower sub-band (8–10 Hz) among the two groups. No globally dysfunctional MNS in ASD. |
| [64] | 46 participants (23 ASD and 23 controls) age 22–46 | EEG | No significant variation between ASD and controls in P3 ERP component. Larger number RP (readiness potential) Laplacian both in congruent and incongruent trials in ASD. No effect of intended action on early visual processing detected. |
| [71] | 33 participants 16 ASD (mean age 16.2) and 17 controls (mean age 16.5) | EEG | Low alpha coherence in central-temporal area of right hemisphere in ASD. Condition of congruence in joint attention perception; no influencing factor for EEG coherence in ASD and controls. No dysfunction in frontal-parietal attention-oriented network of adolescent ASD. Support of theory of underconnectivity in ASD. |

## 4. Conclusions

ToM is a multifaceted approach, which encompasses a variety of cognitive and neurobiological aspects and has been found to be impaired in individuals with ASD. One of the theories that attempts to account for some of these impairments is that of "broken mirror neurons", indicating dysfunctions in the proper activation of a neural circuit responsible for the efficient perception of motion activity. The aberrant firing of this neuronal circuit is suggested to have a negative impact on the ability to encode the intentions behind observed actions and further burdens the mechanism that underlies imitation, joint attention, empathy and ToM in ASD. The present review examined the most recent available studies, in particular studies conducted within the past 15 years, with respect to the connection between the function of MN in individuals with ASD and ToM-reflecting sensorimotor, social and attentional stimuli. The neuroimaging studies reviewed in this paper examined the modulation of attenuation of the *mu* rhythm in ASD using EEG screening tests as a marker for measuring MNS activity. The majority of them approached the theory of broken mirrors critically; the results, however, are contradictory, presenting divergent findings in terms of *mu* suppression and its relation to the performance of individuals with ASD in the experimental tasks of these studies. This deviation may be attributed to the large variation of phenotypical symptomatology across the spectrum of autism, as well as to the limitation of the methodological approaches of the research studies, such as limited sample numbers, a restriction to examining only specific cerebral areas, as well as an inadequate connection of the *mu* suppression emergence to other cognitive operations. Nevertheless, the review revealed discrepancies in the function of MNS in ASD, despite the fact that the activity of this neural network is differently interpreted by the researchers of each study. A clear pattern of aberrant *mu* suppression in ASD is, however, indicated in the reviewed studies, without it being exclusively attributed to dysfunctional MN. The role of MN or cerebral motor activation in general has been challenged, even in neurotypically developing infants. The study of Southgate and Begus [74] showed that nine-month-old children demonstrated motor activation in anticipation of an action, regardless of whether the action was within their own skillset of movement or not. More particularly, the study demonstrated independence in terms of coupling the perceived action with a matching motion representation, indicating that the suppression of the alpha wave is linked to action prediction but that it does not necessarily indicate the activation of MN. These findings were interpreted in alignment with the findings of the study of Kilner et al. [75], which demonstrated that MN are involved not only in the observation of an action but

also in the anticipation of a motion of another person, which facilitates the prediction of intended action goals before the execution of the action itself. The common outcome deriving from this review is that individuals with ASD exhibit deficits in ToM-related cognitive processes, such as the perception and mentalisation of actions in terms of observation execution and imitation, especially under conditions of unfamiliar social engagement. Impairments in the interpretation of social cues further burden social communication in ASD. It is worth mentioning, however, that the findings of this review suggest a relation between low performance in mentalising tasks, which is nevertheless not correlated to autistic traits. It would therefore be of particular interest to investigate *mu* suppression as a neurophysiological operation and the way in which it is linked to mechanisms such as mentalising. It is crucial to conduct further research, in order to gain a more conclusive insight regarding the mechanisms underlying the connection between ToM and neurophysiological operations.

**Author Contributions:** Conceptualization, M.A.; investigation, M.A. and V.S.; writing—original draft preparation, M.A. and V.S.; writing—review and editing, M.A. and V.S.; supervision, M.A. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was part of the SFB 1252 "Prominence in Language" in the project C03 "Reference management in bilingual narratives" (Principal Investigators: Prof. Christiane Bongartz and Prof. Jacopo Torregrossa) at the University of Cologne and was funded by the German Research Foundation (DFG).

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Review*

# The Effects of the Early Start Denver Model for Children with Autism Spectrum Disorder: A Meta-Analysis

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Received: 16 May 2020; Accepted: 9 June 2020; Published: 12 June 2020

**Abstract:** This meta-analysis examined the effects of the Early Start Denver Model (ESDM) for young children with autism on developmental outcome measures. The 12 included studies reported results from 640 children with autism across 44 unique effect sizes. The aggregated effect size, calculated using a robust variance estimation meta-analysis, was 0.357 ($p = 0.024$), which is a moderate effect size with a statistically significant overall weighted averaged that favored participants who received the ESDM compared to children in control groups, with moderate heterogeneity across studies. This result was largely driven by improvements in cognition ($g = 0.412$) and language ($g = 0.408$). There were no significant effects observed for measures of autism symptomology, adaptive behavior, social communication, or restrictive and repetitive behaviors.

**Keywords:** autism; early intervention; Early Start Denver Model

## 1. Introduction

The estimated prevalence of autism spectrum disorders (ASD) has continuously increased in recent decades with the most current prevalence rates estimating that 1 in 54 children under 8 years of age are diagnosed with ASD [1]. This includes an increasing prevalence of young children being diagnosed partly due to the more widespread use of early screening measures and adaptations to diagnostic tools that has led to children being diagnosed with ASD as early as 12–18 months [2]. Children this young need early intervention services that have been designed for and tested with them, given the many developmental and social-emotional differences of infants and toddlers when compared to preschoolers and older children [3]. Given the increasing prevalence estimates of ASD and the high cost of ASD treatments [4], it is critical to identify ASD intervention approaches that are appropriate and effective for supporting young children and their families.

### *1.1. Naturalistic Developmental Behavioral Interventions*

Naturalistic developmental behavioral interventions (NDBIs) are one class of ASD interventions that are particularly geared towards the needs of young children [5]. The term NDBI describes interventions that use strategies involving naturally–occurring environments and activities, child-responsive interaction styles, and teaching content and strategies derived from developmental science as well as the science of applied behavior analysis.

In a recent systematic review and meta–analysis of early interventions for children with ASD, Sandbank and colleagues [6] identified a subset of 26 group design studies that examined the effects of NDBIs and found that the NDBIs showed the strongest body of evidence compared to the other

intervention types included. However, the NDBI studies had multiple methodological and quality limitations across them, especially where 47.59% used outcome measures that were proximal to the intervention goals and 78.77% measured outcomes in contexts similar to the intervention context. Previous reviews have indicated that studies that use proximal and context-bound measures likely inflate intervention effects [7,8]. Additionally, 47.09% used outcome measures at risk of correlated measurement error (CME) due to the participation of adults in outcome measurement who have been trained in the intervention strategies. Sandbank and colleagues found that the group of 26 NDBIs resulted in significant improvements in social communication, cognition, play, and language, but, when examining results from only those studies that did not rely on a parent report (a measurement type that is susceptible to CME), only play and social communication outcomes showed significant improvements.

### *1.2. Early Start Denver Model*

The Early Start Denver Model (ESDM) is an NDBI specifically designed for the needs of very young children with ASD that has been widely studied [9]. The ESDM is one of the few comprehensive early intervention programs for ASD. Although it has a particular focus on autism-specific impairments, it teaches skills across nine developmental domains. The ESDM, which is one of the few commercially available NDBIs, has previously been identified as a promising and cost-effective intervention [10] and has been examined in two systematic reviews. The first review included 15 studies using a variety of study designs [11] and reported overall positive results. However, over half of the included studies had methodological weaknesses. A second review [12] of 10 studies found similar findings and reported that, although most of these studies had positive results, the three comparative studies had mixed findings. Problems of study quality in both meta-analyses included lack of true experimental designs, lack of blind assessment, and small sample sizes.

The purpose of this meta–analysis was to expand and improve upon the findings of these previous reviews in several ways: by including many more recently published studies, by using a meta-analytic approach that allowed for a quantitative understanding of effects, by focusing on comparative studies, and by examining effects on specific domains as well as overall effects of the intervention. This would help identify strengths and areas needing improvement for a well–known early ASD intervention.

### *1.3. Research Questions*

This systematic review and meta-analysis of the effects of the ESDM on outcomes for young children with ASD was conducted to address the following questions: (1) Does the ESDM result in significant improvements in outcomes for young children with ASD, both overall and specifically in the domains of autism symptomology, language, cognition, social communication, adaptive behavior, and repetitive behaviors? (2) Are the findings affected by quality and study design features, including proximity and boundedness of measurement?

## 2. Materials and Methods

### *2.1. Eligibility Criteria*

Eligibility criteria are presented in Table 1. Studies were included in the meta-analysis if the study enrolled participants with ASD or at risk for ASD under age 6. The intervention type was restricted to the ESDM, but could include individual, group, or parent-implemented ESDM, or interventions that were derived from ESDM (e.g., Infant Start [13]). Study design was restricted to group comparison studies (randomized control trials or quasi experimental designs). Included studies were required to have a non–ESDM treatment comparison group, which could include: treatment as usual, waitlist control, or parent education only, or a treatment comparison that did not include ESDM interventions. Studies that did not have a comparison group (e.g., single case design or pre/post design) were excluded. Studies had to report at least one child outcome that provided adequate information to calculate a standardized mean difference effect size (e.g., means and SDs or F statistics).

Studies had to be published in English to be eligible for inclusion due to the language restraints of the coders. Follow-up studies were excluded as the only data from the timepoint closest to the end of the intervention.

**Table 1.** Inclusion criteria and search terms.

| Inclusion Criteria | Criteria | Corresponding Search Terms |
|---|---|---|
| Participants | Autism spectrum disorder, all participants younger than age 6 | "Early Start Denver Model" (Anywhere) assign* OR group OR BAU OR "wait list" OR RCT OR random* OR quasi OR control* OR trial (Abstract) |
| Intervention | Early Start Denver Model | |
| Comparison | Treatment as usual, waitlist control, general information only, referral to other services, or non-ESDM intervention | |
| Outcome | Any child outcome | |
| Study Design | Group design study, including randomized control trial and quasi experimental design | |

### 2.2. Search Procedure

A total of nine databases were searched through Proquest: (American Psychological Association (APA) PsycArticles, APA PsycInfo, APA PsycTests, Dissertations and Theses at the University of California, Education Resources Information Center (ERIC), Linguistics and Language Behavior Abstracts, PAIS, ProQuest Dissertations and Theses A&I, Sociological Abstracts). The final search was completed in October 2019. Unpublished or "gray" literature was searched using the online databases of dissertations and theses as well as proceedings from relevant conferences (e.g., International Society for Autism Research) and reference lists. The search and study selection process were completed by the first author.

### 2.3. Data Extraction and Coded Variables

All child outcome measures that were reported were recorded from each study. If a study reported both a total or overall score and subscale scores, only the total/overall score was used. However, the subscale scores were used for the appropriate outcome–specific meta–analysis. For example, if a study reported both the overall developmental quotient from the Mullen Scales of Early Learning (MSEL) and the subscales, the overall score was used in the overall outcome analysis, and the expressive and receptive language subscales were used in the language outcomes analysis [14]. Only outcomes from the timepoint most proximal to the end of the intervention were included.

Study-level characteristics were recorded, including the location in which the study took place, length of intervention delivery (in weeks), intensity of delivery (hours per week), mean child age (in years), percent of participants that were male, the primary person implementing the intervention (parent or professional, which included researcher, teacher, or therapist), whether the intervention included a parent training component, the format of the intervention delivery (individual, group, or mixed), and the fidelity of the intervention implementation, if reported.

Study quality indicators were recorded, including the use of random assignment and the use of assessors who were blind or naïve of the group assignment. The measurement–quality variables were coded using definitions and flowcharts described in Sandbank and colleagues [6]. Each measure was coded according to the proximity and context of the measure.

*Measurement proximity.* Proximity of the measurement was coded as distal or proximal. Distal measures were defined as those behaviors measured using developmentally–scaled tests meant to measure general development. Proximal measures were defined as those in which the measurement directly measured the goals of the intervention. For example, the MSEL would be considered a distal measure, whereas a child's ability to imitate would be considered a proximal measure since this is a behavior that is specifically targeted in the ESDM curriculum.

*Measurement context.* The context of measurement was coded as generalized or context bound. Generalized outcomes were defined as outcomes that were measured in a context differing from the intervention context of at least one dimension (setting or interaction partner). Context–bound measures (CME) were defined as those that were taken in the same context as the intervention was delivered. For example, measuring a child's language using a subscale of the MSEL would be coded as generalized because it uses different materials, interaction styles, and a likely interaction between the partner and setting, whereas measuring a child's language during an intervention session with their usual therapist would receive a context-bound code. Parent questionnaires were coded as generalized because they are intended to capture the child's generalized tendency to behave in the home context. The use of parent/teacher reports was also coded. Potential for CME was defined as any measure involving an adult trained in the intervention. This included a parent report if and only if the parent had been trained in the intervention.

### 2.4. Analytic Strategies

The standardized mean difference effect size was calculated using Hedges' g to compare group differences (treatment vs. control) at post-test. Hedges' $g$ corrects the slight bias in Cohen's $d$ that occurs in studies with small sample sizes, and is, therefore, a more conservative estimate of effect in a sample of studies with high variability [15]. When studies did not report means and standard deviations, the effect size was calculated from an F-statistic, derived from a group*time ANOVA to mitigate the concern of effect-size inflation [16].

A robust variance estimation (RVE) meta-analysis was conducted using the robumeta package on R [17]. The RVE meta–analysis accounts for the nesting of multiple effect sizes within one study [18]. This method was selected rather than traditional meta-analyses, which use only one effect size per study, to account for the fact that the ESDM targets a variety of skills and its efficacy is generally assessed using more than one outcome measure. Separate meta analyses were conducted for each subskill analysis using separate RVE meta-analyses. Meta-regression analyses were conducted to understand the contributing factors of study-level characteristics (dose and person implementing) and study quality indicators. The heterogeneity of effect sizes was examined using $\tau^2$ and $I^2$. Between study variance represented by $\tau^2$, which is in the metric of the effect size. $I^2$ represents the percent of variability that is true heterogeneity across the observed effect estimates. Higher levels of $I^2$ indicate greater dispersion between effect sizes that may be accounted for with moderator analyses [19]. A $p < 0.05$ alpha level was selected as the level of significance for all analyses.

A primary coder (the first author) read and extracted the data from all studies. A second person independently extracted the data from each study so that all variables on 100% of the included studies were coded by two raters. Overall reliability of independent ratings across all coded measures was 97.2%. Disagreements were resolved by first verifying the information in the manuscript and then by discussing between coders, if needed, until agreement was reached so that 100% agreement on all variables was reached. All statistical analyses were completed using the verified data set.

Although efforts were made to minimize publication bias by including gray literature searches, analyses were included to detect bias. Publication bias was examined through visual analysis of a funnel plot and the Egger's test of a small study bias [20].

## 3. Results

### 3.1. Study Selection

The initial search identified 411 articles to be screened for inclusion. After the initial and full-text screening of the identified articles, 12 studies, including 11 published manuscripts and one dissertation [21], were included in the final analysis. A Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram of exclusion procedures is provided in Figure 1.

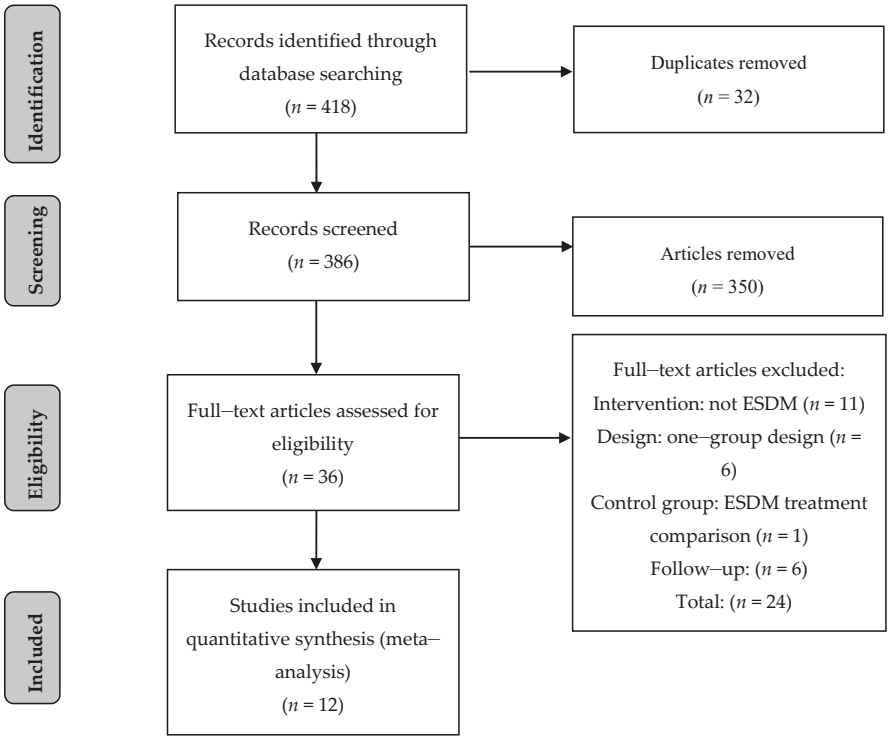


**Figure 1.** Prisma diagram of study inclusion.

### 3.2. Study Characteristics

The 12 included studies were published between 2010 and 2019. The studies took place in five different countries: Australia, Austria, China, Italy, and the United States. The studies included 640 participants (286 intervention and 354 control). The participants ranged in age from nine months to five years old with an average overall age of 2.51 years (SD = 0.89). The studies that reported on gender reported that 80.6% of the samples were male. A total of 44 different effect sizes were reported across the 12 studies. A range of outcome measures were used. Overall study characteristics are shown in Table 2 and characteristics specific to each effect size are shown in Table 3.

In five studies, the parent was the sole agent of implementation. An additional five studies used an intervention approach that incorporated parent coaching but was primarily implemented by a professional. Four studies used a group-based approach: two studies trained parents in groups [21,22] and two studies used group-delivered ESDM [23,24]. Outcomes of studies that included parents did not show significantly higher outcomes than those that did not (B = 0.289, $p = 0.39$). Overall fidelity of implementation was high (mean = 83.2%, range = 75–92%). The studies used a wide range of intervention dosages both in intensity and in length, ranging in intensity from one hour per week to 20 hours per week, and ranging in length from six weeks to 156 weeks. This resulted in total hours of intervention ranging from 12 hours to 2080 hours. However, a meta-regression showed that child outcomes were not significantly related to the length of intervention (B = −0.01, $p = 0.46$), to the hours per week of intervention (B = −0.02, $p = 0.73$), or to the total number of hours (B = 0.004, $p = 0.66$). Additional information about what interventions the control groups received during the study period is included in the Table A1 (Appendix A).

**Table 2.** Summary descriptions of included studies.

| Author (year) [ref] | Country | Participants (*n*) | Average Age (Years) | Percent Male | Intervention Length (Weeks) | Hours per Week | Primary Implementer | Parent Coaching | Group | Fidelity | Blind Assessors | Random Assignment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dawson (2010) [25] | USA | 48 | 1.95 | 72 | 104 | 20 | Professional | yes | no | 85% | yes | yes |
| Rogers (2012) [26] | USA | 98 | 1.75 | 77.55 | 12 | 1 | Parent | yes | no | 75% | yes | yes |
| Rogers (2014) [13] | USA | 11 | 0.75 | 65.63 | 18 | 1 | Parent | yes | no | 91% | yes | no |
| Vivanti (2014) [23] | Australia | 57 | 3.4 | 87.72 | 52 | 15 | Professional | yes | yes | 92% | no | no |
| Fox (2018) [21] | USA | 10 | 2.73 | 70 | 6 | 3 | Parent | yes | yes | | NA | yes |
| Zhou (2018) [22] | China | 43 | 2.21 | 88.37 | 26 | 1.5 | Parent | yes | mix | 80% | yes | no |
| Xu (2018) [27] | China | 40 | 3.77 | 88.75 | 8 | 5 | Professional | yes | no | 85% | yes | yes |
| Colombi (2018) [28] | Italy | 92 | 2.76 | | 24 | 6 | Professional | no | no | 80% * | no | no |
| Vinen (2018) [24] | USA | 59 | 3.11 | 88.1 | 156 † | 15–20 | Professional | yes | yes | 80% * | yes | no |
| Vismara (2018) [29] | USA | 30 | 2.46 | 70.83 | 12 | 1.5 | Parent | yes | no | | yes | yes |
| Holzinger (2019) [30] | Austria | 16 | 3.62 | 100 | 52 | 4.6 | Professional | no | no | 80% * | no | no |
| Rogers (2019) [31] | USA | 118 | 1.72 | 77.97 | 116 | 16 | Professional | yes | no | 84% | yes | yes |

*Note.* † Indicates an average duration. * Indicates a minimum level of fidelity.

**Table 3.** Summary descriptions of included effect sizes.

| Study Author (Year) [ref] | Outcome Measure | Post-Test Mean (SD): Intervention Group | Post-Test Mean (SD): Control Group | Hedges' *g* (SE) | Distal | General-ized | Parent Report | CME |
|---|---|---|---|---|---|---|---|---|
| Dawson (2010) [25] | ADOS ASD severity | 7 (1.9) | 7.3 (1.8) | 0.16 (0.28) | Yes | Yes | No | No |
| | MSEL ELC | 78.6 (24.2) | 66.3 (15.3) | 0.60 (0.29) | Yes | Yes | No | No |
| | VABS Composite | 68.7 (15.9) | 59.1 (8.8) | 0.73 (0.29) | Yes | Yes | Yes | Yes |
| | Repetitive Behavior Scale | 16.7 (13.1) | 22.0 (16.3) | 0.35 (0.29) | Yes | Yes | Yes | Yes |
| Rogers (2012) [26] | ADOS Modified Social Affect | 26.6 (10.1) | 27.3 (10.6) | 0.07 (0.20) | Yes | Yes | No | No |
| | MSEL DQ | 69.8 (17.9) | 67.9 (17.9) | 0.11 (0.20) | Yes | Yes | No | No |
| | MCDI: Phrases Understood | 12.7 (9.11) | 14.8 (8.1) | −0.23 (0.20) | Yes | Yes | Yes | Yes |
| | MCDI: Vocabulary Comprehension | 106.5 (96.8) | 125.7 (106.4) | −0.19 (0.20) | Yes | Yes | Yes | Yes |
| | MCDI: Vocabulary Produced | 42.3 (62.0) | 38.9 (73.7) | 0.05 (0.20) | Yes | Yes | Yes | Yes |
| | MCDI: Total Gestures | 28.02 (12.6) | 29.8 (13.5) | −0.13 (0.20) | Yes | Yes | Yes | Yes |
| | VABS Composite | 77.4 (9.6) | 80.3 (11.3) | −0.27 (0.20) | Yes | Yes | Yes | Yes |
| | ADOS RRB | 4.0 (1.9) | 3.8 (2.0) | −0.07 (0.20) | Yes | Yes | No | No |
| | Imitative Sequence | 4.6 (3.5) | 3.8 (3.4) | 0.24 (0.20) | No | Yes | No | No |
| | Mean Social Orienting | 0.5 (0.3) | 0.4 (0.4) | 0.13 (0.20) | No | Yes | No | No |
| | Mean Non-Social Orienting | 0.7 (0.3) | 0.6 (0.4) | 0.42 (0.20) | No | Yes | No | No |
| | Mean orient to Joint Attention | 0.3 (0.3) | 0.3 (0.3) | 0.00 (0.20) | No | Yes | No | No |

**Table 3.** *Cont.*

| Study Author (Year) [ref] | Outcome Measure | Post-Test Mean (SD): Intervention Group | Post-Test Mean (SD): Control Group | Hedges' *g* (SE) | Distal | General-ized | Parent Report | CME |
|---|---|---|---|---|---|---|---|---|
| Rogers (2014) [13] | ADOS ASD severity | 3.3 (3.4) | 6.3 (3.9) | 0.75 (0.59) | Yes | Yes | No | No |
| | MSEL Language | 92.4 (29.5) | 45.6 (20.3) | 1.60 (0.67) | Yes | Yes | No | No |
| | MSEL Visual reception | 96.1 (16.4) | 78.7 (9.3) | 1.10 (0.62) | Yes | Yes | No | No |
| Vivanti (2014) [23] | ADOS ASD Severity | 6.9 (2.3) | 6.1 (1.6) | −0.37 (0.26) | Yes | Yes | No | No |
| | MSEL DQ | 67.2 (20.2) | 56.3 (22.5) | 0.50 (0.27) | Yes | Yes | No | No |
| | VABS Composite | 72.1 (13.5) | 73.0 (15.5) | −0.06 (0.26) | Yes | Yes | Yes | Yes |
| Fox (2018) [21] | MCDI: Vocabulary Produced | 164.4 (188.2) | 124.2 (140.7) | 0.22 (0.57) | Yes | Yes | Yes | Yes |
| Zhou (2018) [22] | ADOS ASD Severity | 6.3 (1.3) | 6.6 (1.1) | 0.23 (0.30) | Yes | Yes | No | No |
| | CSBS Total | 40.9 (8.1) | 29.1 (10.3) | 1.27 (0.33) | Yes | Yes | Yes | Yes |
| | GDS Language | 68.6 (22.1) | 37.7 (22.5) | 1.36 (0.33) | Yes | Yes | No | No |
| | GDS Motor | 80.3 (15.1) | 71.7 (13.3) | 0.59 (0.31) | Yes | Yes | No | No |
| | GDS Personal-Social | 74.5 (10.6) | 51.0 (10.8) | 2.16 (0.38) | Yes | Yes | No | No |
| | GDS Eye-hand | 76.0 (13.3) | 56.1 (12.4) | 1.52 (0.34) | Yes | Yes | No | No |
| | GDS Performance | 75.2 (10.3) | 58.9 (15.1) | 1.25 (0.33) | Yes | Yes | No | No |
| Xu (2018) [27] | ASD CARS severity | 30.4 (5.5) | 37.3 (6.7) | 1.09 (0.35) | Yes | Yes | No | No |
| Colombi (2018) [28] | GDS Total | | | 0.37 (0.24) | Yes | Yes | No | No |
| | VABS Composite | | | 0.28 (0.24) | Yes | Yes | Yes | No |
| Vinen (2018) [24] | ADOS ASD Severity | 8.0 (2.6) | 7.8 (2.1) | −0.09 (0.26) | Yes | Yes | No | No |
| | WASI FSIQ | 76.1 (20.8) | 82.8 (18.5) | −0.34 (0.24) | Yes | Yes | No | No |
| Vismara (2018) [29] | Imitation | 1.4 (1.0) | 0.9 (0.8) | 0.48 (0.41) | No | No | Yes | Yes |
| Holzinger (2019) [30] | PDDBI | 41.7 (13.1) | 40.0 (10.7) | −0.13 (0.47) | Yes | Yes | Yes | No |
| | Austrian CDI | 324.7 (201.9) | 193.8 (229.5) | 0.57 (0.48) | Yes | Yes | Yes | No |
| | MSEL DQ | 63.5 (20.2) | 50.0 (19.5) | 0.64 (0.49) | Yes | Yes | No | No |
| | VABS Daily Living | 81.9 (17.4) | 85.4 (13.5) | −0.21 (0.47) | Yes | Yes | Yes | No |
| Rogers (2019) [31] | ADOS ASD severity | 6.7 (2.0) | 6.2 (2.5) | −0.22 (0.18) | Yes | Yes | No | No |
| | Response to joint attention | 76.1 (26.9) | 70.7 (36.6) | 0.17 (0.18) | No | Yes | No | No |
| | MSEL DQ | 83.1 (26.1) | 79.1 (25.6) | 0.15 (0.18) | Yes | Yes | No | No |
| | VABS Composite | 39.8 (12.1) | 36.7 (14.3) | 0.23 (0.18) | Yes | Yes | Yes | Yes |

*Note.* ADOS: Autism Diagnostic Observation Schedule, RRB: Restrictive and Repetitive Behavior, MSEL: Mullen Scales of Early Learning, DQ: Developmental Quotient, ELC: Early Learning Composite, MCDI: MacArthur Bates Communicative Development Inventory, VABS: Vineland Adaptive Behavior Scales, CSBS: Communication and Symbolic Behavior Scales, GDS: Griffith Developmental Scales, CARS: Childhood Autism Rating Scale; WASI FSIQ: Wechsler Abbreviated Scale of Intelligence Full Scale Intelligence Quotient, PDDBI: Pervasive Developmental Disorder Behavior Inventory, CME: Correlated Measurement Error.

### *3.3. Overall Outcomes*

Figure 2 shows the results of the RVE meta–analysis examining the effects of the ESDM on all included outcome measures. The effect size weight is shown for each of the 44 outcome measures arranged by the study. Larger black boxes around the effect sizes represent larger weights in the meta–analysis, and bars represent the confidence intervals. The RVE aggregated effect size resulted in an overall effect size of $g = 0.357$ ($p = 0.024$). This moderate and statistically significant effect size suggests a significant advantage for children who received the ESDM intervention compared to children enrolled in control groups. However, a moderate amount of between–study heterogeneity was observed in this analysis ($I^2 = 64.84\%$, $\tau^2 = 0.16$). The majority of studies showed confidence intervals that overlapped with zero, which indicated that the RVE aggregated effect was driven by a few studies or by specific outcome measures. This further assessed the subgroup analyses below.

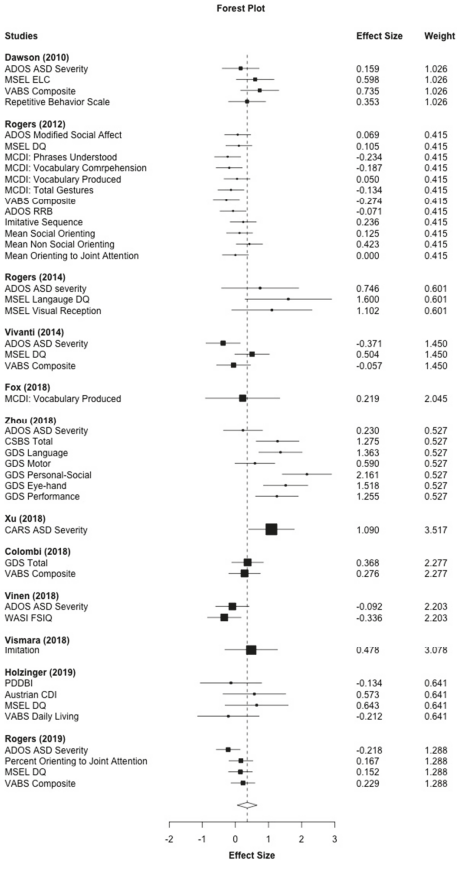


**Figure 2.** Main effect of the Early Start Denver Model (ESDM) intervention on developmental and symptom outcomes. *Note.* ADOS: Autism Diagnostic Observation Schedule, RRB: Restrictive and Repetitive Behavior, MSEL: Mullen Scales of Early Learning, DQ: Developmental Quotient, ELC: Early Learning Composite, MCDI: MacArthur Bates Communicative Development Inventory, VABS: Vineland Adaptive Behavior Scales, CSBS: Communication and Symbolic Behavior Scales, GDS: Griffith Developmental Scales, CARS: Childhood Autism Rating Scale; WASI FSIQ: Wechsler Abbreviated Scale of Intelligence Full Scale Intelligence Quotient, PDDBI: Pervasive Developmental Disorder Behavior Inventory. *Note.* Black boxes indicate the weight of each effect size and bars indicate the confidence interval. The overall effect size is indicated by the open diamond and dotted line ($g = 0.357$).

### *3.4. Study Quality Indicators*

The studies were analyzed for their use of study design elements. Study level quality elements (blind assessors, random assignment) are reported in Table 2, and effect–size specific elements (if the measure was distal, generalized, relied on parent report, or showed potential risk of CME) are reported in Table 3. Thirty-eight of the forty-four (83.2%) elements included measures used developmentally–scaled, distal measures of child outcomes. Forty-three of the included measures (97.7%) used generalized contexts to measure child outcomes. Fourteen outcome measures used parent report measures (31.8%), and 10 outcome measures (22.7%) had potential risk for CME (nine of these 10 studies due to the use of parent report measures). Blind assessors were used in 72.2% of eligible studies (eight out of eleven studies with one study not being included since it only used a parent report so that no assessors were used). Six of the 12 studies (50%) used a randomized study design. A meta regression analysis showed that child outcomes were not significantly associated with distal outcomes (B = 0.28, $p = 0.47$), generalized outcomes (−0.38, $p = 0.20$), parent report (B = −0.08, $p = 0.70$), use of blind assessors (B = 0.15, $p = 0.74$), or the use of a random assignment (−0.02, $p = 0.95$). Furthermore, the inclusion of these variables did not account for the observed heterogeneity ($I^2 = 76.22\%$, $\tau^2 = 0.26$). Because of the high overlap between the use of parent measures and the potential risk of CME, only the variable for the use of parent measures was retained in the meta-regression analysis.

### *3.5. Autism Symptoms*

Figure 3 displays the forest plot for the 10 autism symptomology outcomes that were reported across nine studies. The effect sizes are represented such that positive values indicate a reduction in autism symptomology. The aggregated effect size was $g = 0.070$ ($p = 0.616$), which indicated that children who received ESDM treatment did not show significant improvements in autism symptomology when compared to the control group. A moderate level of heterogeneity was observed ($I^2 = 48.90\%$, $\tau^2 = 0.073$).

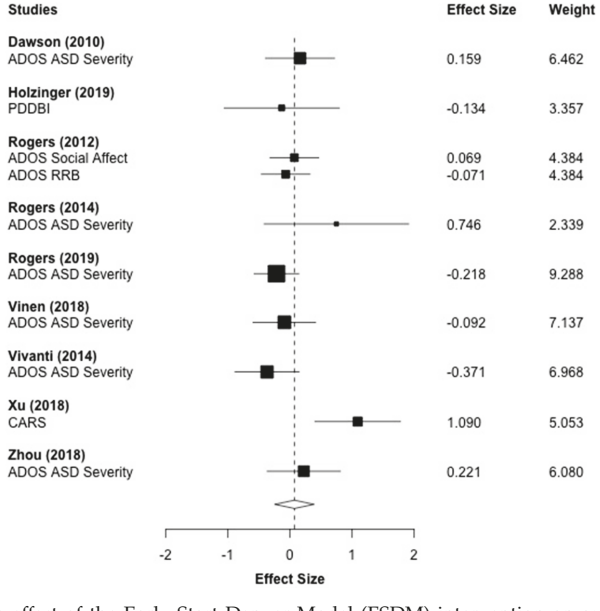


**Figure 3.** Main effect of the Early Start Denver Model (ESDM) intervention on autism spectrum disorder (ASD) symptomology outcomes. *Note.* ADOS: Autism Diagnostic Observation Schedule, RRB: Restrictive and Repetitive Behavior, CARS: Childhood Autism Rating Scale; PDDBI: Pervasive Developmental Disorder Behavior Inventory.

### *3.6. Language*

Figure 4 displays the forest plot for the 19 language outcomes that were reported across 11 studies. The effect sizes represent both expressive and receptive language outcomes. The aggregated effect size was $g = 0.408$ ($p = 0.011$), which indicates that children who received the ESDM intervention made significant progress in language development compared to children in the control groups. A moderate level of heterogeneity was observed ($I^2 = 52.70\%$, $\tau^2 = 0.088$).

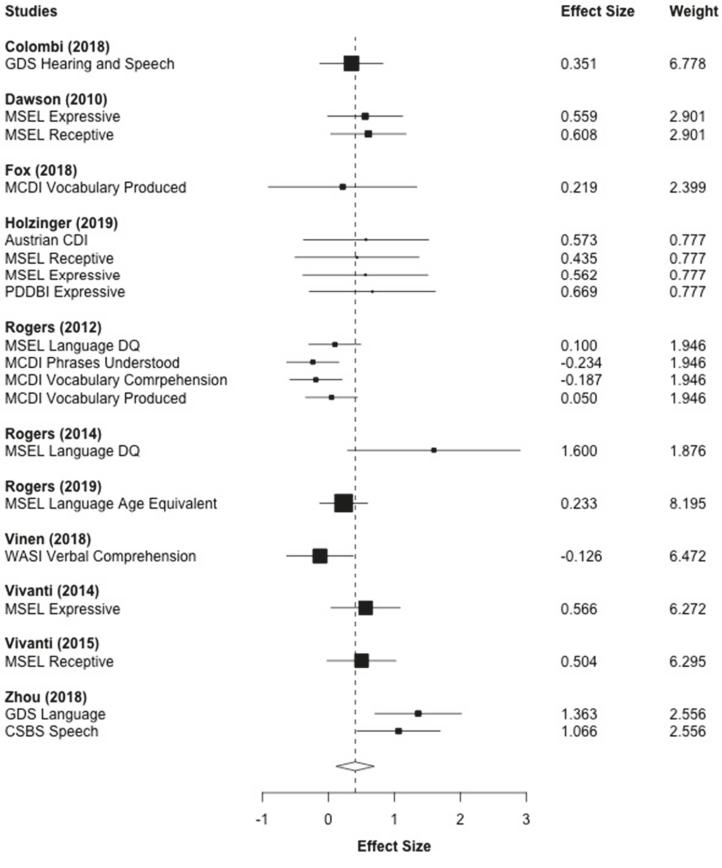


**Figure 4.** Main effect of ESDM intervention on language outcomes. *Note.* MSEL: Mullen Scales of Early Learning, DQ: Developmental Quotient, MCDI: MacArthur Bates Communicative Development Inventory, GDS: Griffith Developmental Scales, WASI: Wechsler Abbreviated Scale of Intelligence, PDDBI: Pervasive Developmental Disorder Behavior Inventory.

### *3.7. Cognition*

Figure 5 displays the forest plot for the 13 cognitive outcomes that were reported across nine studies. The aggregated effect size was $g = 0.412$ ($p = 0.038$), which indicated that children who received the ESDM intervention made significant progress in cognitive development compared to children in the control group. A moderate level of heterogeneity was observed ($I^2 = 66.30\%$, $\tau^2 = 0.145$).

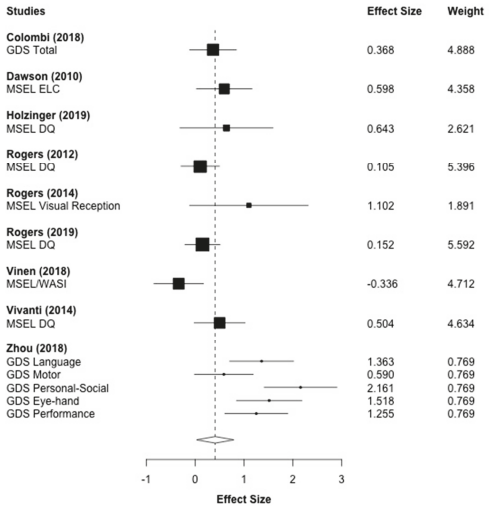


**Figure 5.** Main effect of ESDM intervention on cognitive outcomes. *Note.* MSEL: Mullen Scales of Early Learning, DQ: Developmental Quotient, ELC: Early Learning Composite, GDS: Griffith Developmental Scales; WASI FSIQ: Wechsler Abbreviated Scale of Intelligence.

### *3.8. Social Communication*

Figure 6 displays the forest plot for the 19 social communication outcomes that were reported across eight studies. This included related sub-scores of the Vineland (Communication and Socialization) [32]. The aggregated effect size was $g = 0.209$ ($p = 0.285$), and was not statistically significant. A high amount of heterogeneity was observed across social communication measures ($I^2 = 72.53\%$, $\tau^2 = 0.176$).

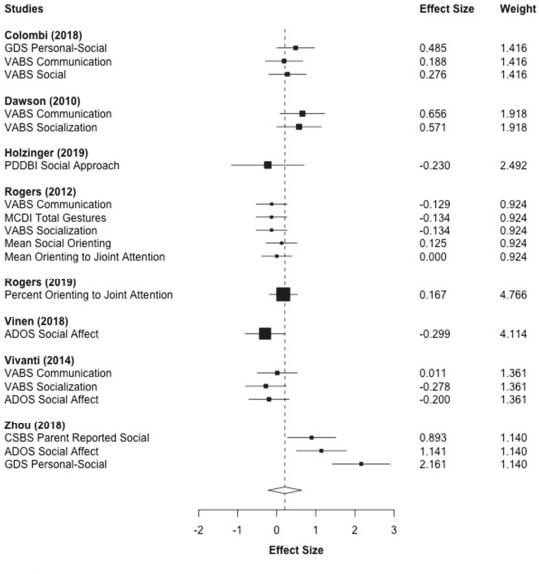


**Figure 6.** Main effect of ESDM intervention on social communication outcomes. *Note*. ADOS: Autism Diagnostic Observation Schedule, MCDI: MacArthur Bates Communicative Development Inventory, VABS: Vineland Adaptive Behavior Scales, CSBS: Communication and Symbolic Behavior Scales, GDS: Griffith Developmental Scales.

### *3.9. Adaptive Functioning*

Figure 7 displays the forest plot for the six adaptive functioning outcomes that were reported across six studies. All of the included effect sizes were taken from the Vineland [32]. The aggregated effect size was $g = 0.121$ ($p = 0.458$), which was not statistically significant. A moderate amount of between-study heterogeneity was observed ($I^2 = 49.03\%$, $\tau^2 = 0.062$).

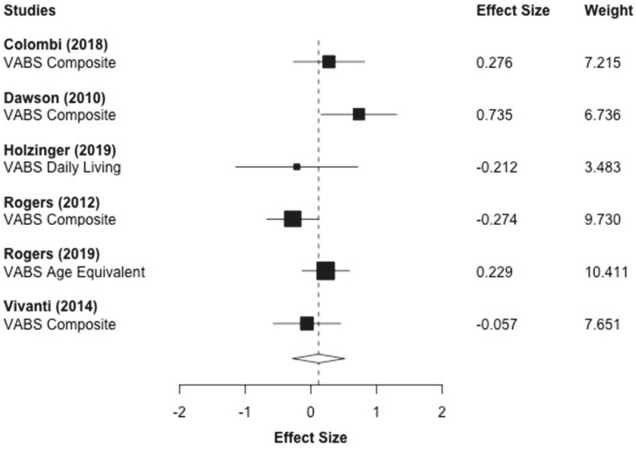


**Figure 7.** Main effect of ESDM intervention on adaptive functioning outcomes. ***Note.*** VABS: Vineland Adaptive Behavior Scales.

### *3.10. Repetitive Behaviors*

Figure 8 displays the forest plot for the five repetitive behavior outcomes that were reported across five studies. The effect sizes are represented such that positive values indicate a reduction in repetitive behaviors. The aggregated effect size was $g = -0.016$ ($p = 0.876$), which indicated that children who received ESDM treatment did not show significant improvements compared to the control group in repetitive behaviors. This finding should be taken with caution due to the low number of included effect sizes.

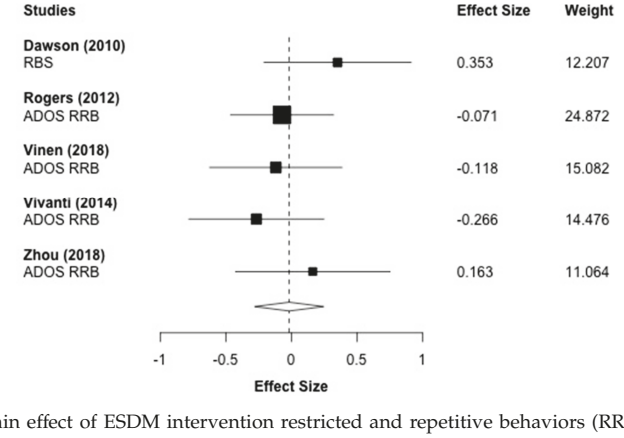


**Figure 8.** Main effect of ESDM intervention restricted and repetitive behaviors (RRB). *Note*: RBS: Repetitive Behaviors Scale; ADOS RRB: Autism Diagnostic Observation Schedule Restricted and Repetitive Behaviors.

### 3.11. Publication Bias

An Egger's test of a small study bias ($p < 0.01$) indicated that there is a risk of a small study bias in this sample. A funnel plot is included in Figure A1 (Appendix B), which shows that two of the 44 effect sizes fall outside of the highlighted area, suggesting a small bias.

## 4. Discussion

This meta-analysis examined the effects of the ESDM for young children with ASD delivered in a variety of formats on a variety of outcomes measures. Across 12 studies that included 44 unique effect sizes, the overall aggregated effect size was $g = 0.357$ ($p = 0.024$). This moderate [33] and statistically significant effect size indicates an overall advantage for children in the ESDM intervention groups compared to children in control groups ($p = 0.024$). (For reference, this represents a gain of 7.84 more points on the Mullen Developmental Quotient than the comparison group.) These significant differences were mostly driven by improvements in cognition ($g = 0.412$) and language ($g = 0.408$). There was a moderate amount of heterogeneity across studies and significant results were not observed for all studies or outcome measures. Nonsignificant differences were observed for the remaining domains: autism symptomology, adaptive behavior, social communication, and restricted and repetitive behaviors (RRBs). Although many of these effect sizes came from one lab, the 12 included studies represent data from five different countries and from interventions of both high and low intensity implemented using a variety of delivery methods including parents, local teachers or therapists, and group-based settings.

One particular strength of this meta-analysis was the general rigor of the measurements used in the included studies. Relatively few measures were at risk of CME, which occurs when measures involve parent interactions with children or parent reports of child skills in studies that have trained parents in the intervention. In the current sample, only 22.7% of studies had potential risk of this source of CME. This is a great deal fewer than the group of NDBI studies that Sandbank and colleagues [6] reported on, which found that 47% of outcomes were at risk for CME. In addition, most studies included in this meta–analysis used norm-referenced measures that were distal (83%) and generalized (97%). This is considerably more than the general pool of NDBI studies included in the Sandbank analysis in which 52% of outcomes used distal measures and 21% used generalized measures. The high rate of distal and generalized measures seen in this current sample of studies reduces concerns of effect size inflation due to the measurement error.

Given the relative strength in the quality of measurements used in the studies included in the current review, the current findings of significant improvements in language and cognition related to the ESDM compare favorably with previous reviews of ASD interventions. Sandbank and colleagues [6] found that, although NDBIs are generally making significant improvements across domains, the improvements in language and cognitive outcomes as a result of NDBIs were mostly smaller in magnitude (language: $g = 0.21$, $p < 0.05$, cognitive: $g = 0.18$, not significant). In comparison, the present analysis showed significant language and cognitive improvements of $g = 0.408$ and 0.412, respectively. The effect sizes for language in the present ESDM study is also larger than the effect size of $g = 0.26$ reported in a recent meta-analysis that examined language outcomes of multiple types of early ASD interventions [34].

### *Limitations and Future Directions*

The most prominent limitation was the heterogeneity observed in this sample. This meta-analysis combined a wide range of study designs, measures, and procedures. Twelve of the 44 outcome measures showed results in the negative direction, and the majority of outcomes had a confidence interval that included zero. Thus, the overall positive effect size should be taken cautiously.

Two of the potential contributors to the observed heterogeneity in this analysis involved dosage and delivery. A wide range of dosage was used across the 12 included studies in terms of length

of intervention and intensity of intervention. Although neither length or intensity of dosage were significantly related to outcome magnitude, this lack of association should be considered with caution. In terms of delivery, five of the studies used a parent-implemented approach. In these studies, the dosage refers to the amount of time the parent was coached rather than the amount of time the parent used the strategies with the child. Four of the studies used a group–based approach. In this case, intensity of individual receipt of intervention is likely different from studies that used a one–on–one delivery approach. Thus, the true dosage of intervention is hard to quantify in some of these studies. Lack of relationship between dosage and outcomes has also been shown in several previous meta–analyses of early interventions [7,34]. Further study is needed to understand the role of dosage in intervention outcomes.

A second limitation was in the scientific rigor of the study designs. While all studies were controlled, half of the included studies used a non-randomized control design. Although the meta-regression indicated that there was not a significant relationship between the use of a non-random design and study outcomes, the negative beta weight indicates that randomized controlled studies had smaller effect sizes than the quasi–experimental studies on average. Many of the quasi-experimental studies were carried out outside of university and lab settings, including community implemented studies [28] in which it was not considered feasible or ethical to implement a randomized design. We included these quasi-experimental controlled studies despite the design limitations to represent findings of real-world applications of the ESDM. Other problems with rigor include use of measures based on parent report, outcome measures that were at risk of a correlated measurement error, and non–blinded assessors (in some of the studies).

A third limitation relates to the subgroup meta-analysis that showed nonsignificant changes on measures of autism symptomology, adaptive behaviors, repetitive behaviors, and social communication. This indicates that the ESDM intervention may be less effective at targeting these characteristics of early ASD. However, in the case of ASD severity and RRBs, this may also be partly due to an issue in measurement. Many of the outcome measures included for these domains came from the Autism Diagnostic Observation Schedule (ADOS) [35]. The ADOS is intended to capture relatively stable characteristics of ASD symptomology including social communication for diagnostic purposes and was not created with the intention of measuring a treatment-related change. A more recent measure, known as the Brief Observation of Social Communication Change (BOSC) [36], was created for this purpose, and may be a more useful tool for capturing change in these outcomes. Future studies should further examine these subdomains using more sensitive outcome measures and should consider additional intervention strategies to specifically target these areas.

A final limitation is the risk of small study bias observed. However, this concern was mitigated by using a correction for small study effects included in the RVE meta-analyses estimation and through extensive searching of gray literature, which included one unpublished study.

## 5. Conclusions

Based on the moderate and significant overall effect size resulting from this meta-analysis involving 640 participants across 12 studies, the ESDM shows promise as an effective practice for young children with ASD in improving outcomes in some areas affected by early ASD, especially language and cognitive outcomes. Domains involving autism symptomology, social communication, adaptive behaviors, and repetitive behaviors did not show an ESDM advantage and may require additional treatment efforts and/or more sensitive outcome measures. This body of evidence has several strengths in scientific rigor including the use of distal and generalized outcome measures and lowered risk of correlated measurement error compared to other NDBI interventions, but also shows a weakness in the number of quasi-experimental non-randomized study designs. Lastly, the studies reported high fidelity of treatment implementation across a variety of delivery contexts, including five different countries, group and individual settings, and a range of implementors that included parents, community therapists, and teachers.

**Author Contributions:** Conceptualization, E.A.F. and S.J.R. Methodology, E.A.F. Validation, K.O. and S.F.V. Formal analysis, E.A.F. Writing—original draft preparation, E.A. Writing—review and editing, E.A.F., K.O., S.F.V., and S.J.R. Supervision, S.J.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received funding from NIH T32MH07312 during the time period 2018-2020. The funding sources were not involved in the study design or implementation.

**Conflicts of Interest:** Sally J. Rogers has received royalties from Guilford Press and honoraria for lectures related to this paper.

## Appendix A

**Table A1.** Description of the control group.

| Author (Year) | Intervention Group (*n*) | Control Group (*n*) | Control Group |
|---|---|---|---|
| Dawson (2010) [25] | 24 | 24 | Treatment as usual, plus intervention recommendations, community referrals, and reading material |
| Rogers (2012) [26] | 49 | 49 | Treatment as usual |
| Rogers (2014) [13] | 7 | 25 | Treatment as usual |
| Vivanti (2014) [23] | 27 | 30 | Group-based "generic" intervention program for children with autism spectrum disorders (ASD) |
| Fox (2018) [21] | 5 | 5 | Waitlist |
| Zhou (2018) [22] | 23 | 20 | Treatment as usual |
| Xu (2018) [27] | 20 | 20 | Eclectic intervention services matching the amount of time the Early Start Denver Model (ESDM) group received |
| Colombi (2018) [28] | 22 | 70 | Treatment as usual |
| Vinen (2018) [24] | 31 | 28 | Group-based eclectic intervention program for children with ASD |
| Vismara (2018) [29] | 16 | 14 | Monthly check-ins and access to online material |
| Holzinger (2019) [30] | 7 | 6 | Treatment as usual |
| Rogers (2019) [31] | 55 | 63 | Treatment as usual |

## Appendix B

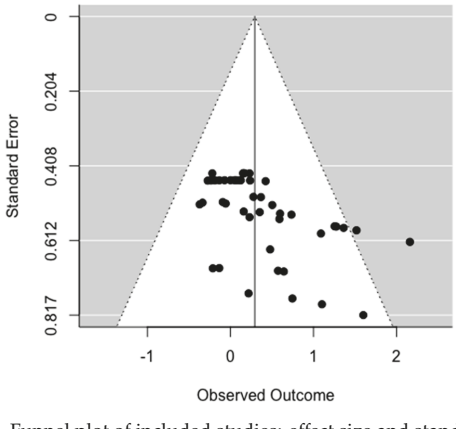


**Figure A1.** Funnel plot of included studies: effect size and standard error.

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*Review*

# Epidemiology of Autism Spectrum Disorders: A Review of Worldwide Prevalence Estimates Since 2014

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Received: 29 March 2020; Accepted: 21 April 2020; Published: 1 May 2020

**Abstract:** The prevalence of Autism Spectrum Disorder (ASD) has increased dramatically in recent decades, supporting the claim of an autism epidemic. Systematic monitoring of ASD allows estimating prevalence and identifying potential sources of variation over time and geographical areas. At present, ASD prevalence estimates are available worldwide, coming either from surveillance systems using existing health and educational databases or from population studies specifically performed. In the present article, we present a review of the ASD prevalence estimates published since 2014. Data confirm a high variability in prevalence across the world, likely due to methodological differences in case detection, and the consistent increase of prevalence estimates within each geographical area.

**Keywords:** prevalence estimate; autism; predictors; surveillance review

## 1. Introduction

In the last decades, a large increase in the prevalence of Autism Spectrum Disorder (ASD) has been observed, generating claims about an "epidemic" of autism [1,2]. Correct estimates of ASD prevalence rates are important, firstlyin order to determine the economic and health services burden of this condition and to allocate sufficient funding and adequate services for children and adults with ASD and their families. A growing population of people with ASD implies the necessity of increased service availability including training of professionals, as well as identification of additional resources that can emerge by the recognition of cases in the population [3]. Furthermore, accurately determining ASD prevalence can help to understand which groups are exposed to disparities in healthcare access for developmental evaluations [4], besides being more at risk for ASD due to geographical and environmental factors [5].

Studies that estimate ASD prevalence result in wide variability of prevalence rates that call for paying attention on possible reasons for the observed changes in prevalence, and advice for caution when claiming that there is an autism epidemic [2,6].

One important source of variation in prevalence estimates are the methodological differences in case definition and case-finding procedures. In particular, some studies are carried out on existing administrative databases such as special education data, health or social records of national registers for case identification, or specific condition registers (defined as "administrative data" when relying on one database, or "multisource" when combining data from multiple databases). Other studies rely on a two-stage or multistage approach to identify cases in underlying populations; the first stage is often based on questionnaire requesting behavioural descriptions or checklist based on DSM, where informants could be in turn teachers, parents or health professionals (defined as "ad hoc studies"). Finally, some studies are surveys based on interviews to parents or teachers, who are required to state if the child presents a condition that can be related to ASD (defined as "reports"). Obviously,

sample size and catching area represent further characteristics of the studies that can affect prevalence estimates [7]. Indeed, surveyed areas vary in terms of service development as a function of the specific educational or health care systems of each country and of the year of the study [6]. Moreover, socio economic factors [8,9] and autism awareness [10] can influence assessment of the case and consequently prevalence estimate.

Case definition is the other challenge that affects prevalence estimate. Diagnostic category (AD, ASD, PDD), as well as age range considered are very important sources of prevalence estimate variation [7]. Changing definitions and labelling practices that change over time, as in the case of the introduction of diagnostic manuals' revisions, can produce change in labelling but also "diagnostic substitution" whereby similar symptoms can be classified under different disabilities during different time periods [11,12]. Lastly, cultural influence can affect the definition of case causing differences in the estimation of prevalence in different ethnic/cultural groups [13,14].

In the present paper, we present a brief narrative review of the most recent ASD prevalence estimates worldwide. We describe evidences according to two main criteria, i.e., the geographical setting and the case-finding procedure of the study. Finally, we attempt to demonstrate if these criteria act as predictive factors for underestimating or overestimating prevalence figures.

## 2. Prevalence Estimates

Many prevalence studies have been performed worldwide since 1966. In 2012, Elsabbagh et al. [7] published a comprehensive review of the studies performed until 2012: these studies differed with respect to diagnostic category, diagnostic criteria, age at prevalence evaluation, extent of the targeted geographical area, and source of data on the diagnoses. These methodological differences, together with the large time span (almost 50 years from the first to the last study included in the review), at least partly account for the large differences observed in the estimated prevalence. Overall, estimates ranged from 0.19/1000 to 11.6/1000. The former estimate refers to the Autistic Disorder (AD), diagnosis based on Rutter's criteria (1978), age range 0–14 years, geographical area of West Berlin (Germany), and on data extracted from the registry of the university clinic of child psychiatry and/or the German Society for Autistic Children (1986). The latter refers to the Pervasive Developmental Disorder (PDD), diagnosis based on ICD-10, age range 9–10 years, geographical area of South Thames (UK), ad hoc study (2006). Taking into account the diagnostic category, the median prevalence estimates were 1.00/1000 for AD (from 0.19/1000 in Germany to 7.26/1000 in Sweden) and 6.16/1000 for PDD (from 3.00/1000 in Denmark to 11.6/1000 in UK). When considering PDD, the median prevalence was similar to the USA overall ASD prevalence estimated in 2000–2002, but much lower than the USA prevalence estimates since 2006.

In 2014, Tsai updated the review by [7], but only negligible differences emerged in the median prevalence estimates, which were confirmed to be 1.32/1000 for AD (from 0.19/1000 in Germany to 7.26/1000 in Sweden) and 6.19/1000 for PDD/ASD (from 3.00 in Denmark 2002 to 12.3/1000 in Netherlands) [15]. The review by Tsai included almost all papers evaluated by [7], specifically 59/59 = 100% for AD and 33/35 = 94.3% for PDD prevalence studies. In his review, Tsai examined 15 and 28 additional studies estimating AD and PDD/ASD prevalence, respectively. Table 1 reports a summary of the results of the reviews by [7,15].

Since the publication of the reviews by Elsabbagh et al. and Tsai, more prevalence studies have been performed worldwide. In the following, we report the prevalence studies published since 2014 according to the geographical area of reference. Some studies yield ASD prevalence estimate at different calendar year and/or in different age classes: where possible, we selected the more recent estimate of prevalence that referred to age 8. The list of studies with details and prevalence estimates are presented in Tables 2–4.

**Table 1.** Summary of PDD prevalence estimates of the studies included in the reviews by [7,15] *.

| | From [7] | | | | From [15] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *N* Studies | Publication Year | Prevalence (/1000) | | *N* Studies | Publication Year | Prevalence (/1000) | | Papers Examined by [15] Already Included in [7] (%) |
| | | Range | Median | Range | | Range | Median | Range | |
| Europe | 14 | 2000–11 | 6.16 | 3.0 to 11.6 | 21 | 2000–12 | 6.19 | 3.0 to 12.3 | 66.7 |
| Middle East | 3 | 2007–12 | 0.63 | 0.14 to 2.9 | 4 | 2007–12 | 1.76 | 0.14 to 24.0 | 75.0 |
| Asia | 4 | 2008–11 | 14.41 | 1.6 to 18.9 | 6 | 2008–12 | 6.50 | 1.4 to 26.4 | 66.7 |
| Australia & New Zealand | 1 | 2004 | 3.92 | – | 2 | 2004–09 | 3.15 | 2.4 to 3.9 | 50.0 |
| North America | 10 | 2001–10 | 6.65 | 3.4 to 11.0 | 24 | 2001–14 | 7.17 | 0.21 to 17.4 | 33.3 |
| Central & South America | 3 | 2008–10 | 2.72 | 1.3 to 5.3 | 4 | 2008–11 | 3.99 | 1.7 to 5.3 | 75.0 |
| Africa | 0 | – | – | – | 0 | – | – | – | |

* [7] presented the prevalence estimates for the diagnostic categories Autistic Disorder (AD) and Pervasive Developmental Disorder (PDD), which is the diagnostic category that evolved to ASD passing from DSM-IV to DSM5; [15] used AD, and PDD or ASD. We report only the prevalence estimates for PDD (or ASD).

**Table 2.** Summary of prevalence studies published since 2014 in Europe and Middle-East.

| Country | Extent of Coverage | Area/Region | Year of Prevalence Estimate | Age (Years) | ASD Detection Based on | Prevalence/1000 | 95% CI | Reference |
|---|---|---|---|---|---|---|---|---|
| ***Europe*** | | | | | | | | |
| Sweden | Regional | Stockholm County | 2011 | 6–12 | multisource | 17.4 | 16.8 to 18.0 | [16] |
| Poland | Regional | West Pomerania WP | 2010–2014 | 4–7 | administrative data | 5.4 | 4.8 to 5.9 * | [16] |
| | | Pomerania P | 2010–2014 | 4–7 | administrative data | 5.2 | 4.8 to 5.7 * | [17] |
| | | Overall WP+P | 2010–2014 | 4–7 | administrative data | 5.3 * | 5.0 to 5.6 * | [17] |
| Germany | National | | 2012 | 6–11 | administrative data | 6.0 | na | [18] |
| Denmark | National | | 2015 | 8 | administrative data | 12.6 | 11.7 to 13.5 | [19] |
| Finland | National | | 2015 | 8 | administrative data | 7.7 | 7.0 to 8.4 | [19] |
| France | Regional | South-West | 2015 | 8 | administrative data | 7.3 | 6.0 to 8.7 | [19] |
| | | South-East | 2015 | 8 | administrative data | 4.8 | 4.0 to 5.6 | [19] |
| Iceland | National | | 2015 | 8 | administrative data | 31.3 | 26.4 to 36.8 | [19] |
| Italy | Regional | Tuscany | 2015 | 7–9 | ad hoc: TN, SCQ, ADOS, clinical assessment | 11.5 | 8.3 to 14.6 | [20] |
| Italy | Regional | Piemonte | 2016 | 6–10 | administrative data | 4.2 | na | Admin regional reports |
| Italy | Regional | Emilia-Romagna | 2016 | 6–10 | administrative data | 4.3 | na | Admin regional reports |
| Italy | Regional | Abruzzo | 2018 | 6–8 | administrative data | 8.0 | 6.0 to 10.0 | [21] |
| *Spain* | *Regional* | *Tarragona* | *na (<2018)* | *3–5* | *ad hoc: 1. CAST, EDUTEA, 2. ADI-R, ADOS* | *15.5* | *8.96 to 22.0* | [22] |
| Spain | Regional | Catalonia | 2017 | 6–10 | administrative data | 11.8 | 11.4 to 12.1 | [23] |
| ***Middle East*** | | | | | | | | |
| Iran | National | | na (≤2016) | 6–9 | ad hoc: K-SADS | 1.1 | 0.4 to 1.7 | [24] |
| Oman | National | | 2011–2018 | 5–9 | administrative data from diagnostic centers | 2.0 | 1.9 to 2.2 | [25] |
| Qatar | National | | 2015–2018 | 6–11 | ad hoc: 1. SCQ, 2. QSS-PTI, clinical assessment, QCC-AF | 11.4 | 8.9 to 14.6 | [26] |
| *Lebanon* | *Regional* | *Beirut and Mount Lebanon* | *2014* | *1.3–4* | *ad hoc: M-CHAT* | *15.3* | *7.7 to 22.9* | [27] |

* Prevalence estimates and/or 95%CIs calculated from information reported in the original papers. Prevalence estimates in children aged less than 5 years are reported in italic.

**Table 3.** Summary of prevalence studies published since 2014 in Asia, Australia &New Zealand.

| Country | Extent of Coverage | Area/Region | Year of Prevalence Estimate | Age (Years) | ASD Detection Based on | Prevalence/1000 | 95% CI | Reference |
|---|---|---|---|---|---|---|---|---|
| ***Asia*** | | | | | | | | |
| *China* | *Regional* | *Tiajin* | *2009–2010* | *1.5–3* | *ad hoc: 1. M-CHAT, 2. DSM IV* | *2.8* | *1.6 to 3.9* | [28] |
| China | Regional | Beijing | na (<2015) | 6–11 | ad hoc: 1. CAST, 2. ADOS and ADI-R; mainstream schools | 11.9 | 3.9 to 19.9 * | [29] |
| China | Regional | Jilin | 2013 | 6–10 | ad hoc: 1. CAST, 2. clinical assessment; mainstream /special schools /other settings | 10.8 | 8.7 to 13.5 | [30] |
| | | Jilin | | 6–10 | ad hoc: 1. CAST, 2. clinical assessment; mainstream schools | 1.5 | 0.5 to 2.4 * | [30] |
| | | Shenzhen | 2013 | 6–10 | ad hoc: 1. CAST, 2. clinical assessment; mainstream schools | 4.2 | 2.0 to 8.9 | [30] |
| | | Jiamusi | 2013 | 6–10 | ad hoc: 1. CAST, 2. clinical assessment; mainstream schools | 1.9 | 1.0 to 3.8 | [30] |
| *China* | *Regional* | *Shenzhen* | *2014* | *3.8–4.8* | *ad hoc: 1. ABC; mainstream kindergartens* | *26.2* | *23.7 to 28.7* | [31] |
| Japan | Regional | | 2015 | 6–9 | parents' report data, SRS | 19.0 | 13.0 to 25.0 | [32] |
| | | | 2015 | 6–9 | teachers' report data, SRS | 93.0 | 72.0 to 118.0 | [32] |
| India | Regional | South (Kerala) | 2011-2012 | 6–10 | ad hoc: screening by questionnaire | 5.0 | 2.5 to 7.6 * | [33] |
| India | Regional | Kolkata | 2013 | 3–8 | ad hoc: 1. SCDC, 2. SCQ, 3. ADO Smainstream and special schools | 2.3 | 0.7 to 4.6 | [34] |
| India | Regional | Northwest (Himachal Pradesh) | na (<2017) | 1–10 | ad hoc:1. ISAA, 2, clinical assessment | 1.5 | 1.1 to 2.0 | [34] |
| Nepal | Regional | Makwanpur district | 2014-2015 | 9–13 | ad hoc: AQ-10 screening tool | 3.4 | 1.6 to 5.2 | [35] |
| *Bangladesh* | *Regional* | *North, Sirajganj district* | *2016* | *1.5–3* | *ad hoc: 1. M-CHAT, 2. DSM IV* | *0.8* | *0.0 to 1.5 ** | [36] |
| *Vietnam* | *Regional* | *North* | *2017* | *1.5–2.5* | *ad hoc: 1. M-CHAT, 2. DSM IV* | *10.8 ** | *9.3 to 12.4 ** | [37] |
| ***Australia & New Zealand*** | | | | | | | | |
| Australia | National | | 2005–2006 | 6–7 | parents' report data on diagnoses | 14.1 * | 10.5 to 17.6 * | [38] |
| | | | 2010–2011 | 6–7 | parents' report data on diagnoses | 25.2 | 20.0 to 30.0 | [38] |

* Prevalence estimates and/or 95%CIs calculated from information reported in the original papers. Prevalence estimates in children aged less than 5 years are reported in italic.

**Table 4.** Summary of prevalence studies published since 2014 in North America.

| Country | Extent of Coverage | Area/Region | Year of Prevalence Estimate | Age (Years) | ASD Detection Based on | Prevalence/1000 | 95% CI | Reference |
|---|---|---|---|---|---|---|---|---|
| *North* | | | | | | | | |
| Canada | Regional | Newfoundland and Labrador | 2008 | 6–9 | multisource | 10.8 | 9.4 to 12.3 | [39] |
| | | Prince Edward Island | 2010 | 6–9 | multisource | 10.0 | 7.6 to 12.9 | [39] |
| | | Southeastern Ontario | 2010 | 6–9 | multisource | 16.2 | 14.5 to 18.1 | [39] |
| Canada | Regional | Quebec | 2014–2015 | 1–17 | multisource | 12.2 | na | [40] |
| USA | Regional | 11 States | 2014 | 8 | multisource, evaluated by DSM-IV | 16.8 | 16.4 to 17.3 | [12] |
| | Regional | 11 States | 2016 | 8 | multisource, evaluated by DSM-V | 18.5 | 18.0 to 19.1 | [41] |
| Mexico | Regional | Leon city, Guanajuato | 2011–2012 | 8 | ad hoc: 1. parents' and teachers' SRS, 2. ADOS and ADI-R | 8.7 | 6.2 to 11.0 | [42] |

### *2.1. Europe*

In Sweden, a prevalence study was performed in 2011 based on data from the Stockholm Youth Cohort (SYC) [16]. SYC is a record-linkage study collecting data longitudinally from 2001 to 2011 on all children from 0 to 17 years of age residing in Stockholm County in any time in the specified period; a multisource case ascertainment methodology was used to assess the presence of an ASD diagnosis. An overall large increase of ASD prevalence was observed between 2001 and 2011. Specifically, in children aged 0–17 years, the prevalence moved from 4.20/1000 in 2001 to 14.4/1000 in 2011, with an increase of almost 250%. This increase was mainly due to the huge increase of the ASD prevalence observed in children/adolescents without intellectual disability (almost +700%, from 1.40/1000 in 2001 to 11.0/1000 in 2011), while the increase of ASD prevalence in children/adolescents with intellectual disability was much lower (about +20%, from 2.80/1000 in 2001 to 3.40/1000 in 2011).

In Poland (West Pomeranian—WP—and Pomeranian—P—regions), Skonieczna-Zydecka and collaborators [17] estimated ASD prevalence in 2010–2014 on children from 0 to 16 years of age based on data obtained from both government and private institutions concerning ASD diagnoses and certificates of disability. The prevalence estimates in children of all ages were similar in the two regions (3.24 vs. 3.76/1000 in WP and P, respectively). In both regions, the highest prevalence was observed in children from 4 to 7 years of age (5.35 and 5.25/1000 in WP and P, respectively), yielding an overall estimate of 5.29/1000 in this age class.

In Germany, Bachmann et al. [18] conducted a study aimed at estimating at a national level the administrative prevalence of ASD in individuals aged up to 24 years, using inpatient and outpatient claims data of National health insurance from 2006 to 2012. The 2012 estimates were used to detect differences in prevalence among age groups. Children from 6 to 11 years of age showed the highest prevalence, estimated at 6.00/1000.

In the European Union, 14 countries have participated to the European project "Autism Spectrum Disorders in Europe (ASDEU)": Spain (programme lead), Austria, Belgium, Bulgaria, Denmark, Finland, France, Iceland, Ireland, Italy, Poland, Portugal, Romania, and United Kingdom. Among the goals of the project, there was the estimation of the prevalence of ASD in children aged 7–9 years in 2015. Four countries estimated the prevalence of ASD in 8 years old children using nationwide registry data (Denmark, Finland, and Iceland) or regional statistics (France); prevalence estimates were very different among countries, ranging from 4.76/1000 in South-Eastern France to 31.3/1000 in Iceland (for details, see Table 2) [19].

Eight countries (Austria, Bulgaria, Ireland, Italy, Poland, Portugal, Romania, and Spain) performed ad hoc studies following a shared protocol that required the participation of schools, teachers and parents. Teachers and parents were required to fill in questionnaires (Teacher's nomination and Social Communication Questionnaire, respectively) in order to screen children at risk of having ASD. The children at risk successively underwent a clinical assessment to confirm (or not) the diagnosis of ASD. Until now, only the results of the ASDEU ad hoc study performed in Italy have been published [20]. This study yielded a prevalence estimate of 7.99/1000 when using just the number of children certified with ASD or with other neurodevelopmental disorders in comorbidity with ASD. This prevalence rose to 10.4/1000 when including children identified through the screening procedure, and to 11.5/1000 based on a probabilistic calculation to adjust for non-responses. This estimate was much higher than those based on regional administrative databases storing data on services provided by Child and Adolescent Mental Health Units in Italy, namely SMAIL in Piemonte and ELEA in Emilia Romagna regions. These regional databases yielded in 2016 prevalence estimates of 4.20 and 4.30/1000 in children aged 6–10 years and 6.20/1000 and 5.50/1000 in children aged 3–5 years (Piemonte and Emilia-Romagna regions, respectively). A more recent regional estimate in 2018 based on administrative data from Abruzzo region yielded a higher prevalence estimate of 7.98/1000 in the age class 6–8 years, and quite similar prevalence estimate of 5.74/1000 in the age class 3–5 years [21]. These data suggest that prevalence estimates based on data extracted from registries built to meet administrative informative needs are on average lower than estimates coming from ad hoc studies,

mainly when a two-phase ascertainment design (screening and diagnosis confirmation) is used. UK data support this insight. The high prevalence observed in 2006 in South Thames, consistent with that estimated in Cambridgeshire in 2003–2004 by a school-based population study (15.7/1000) [43], was very different from the prevalence in children aged 8 years estimated by administrative data from the UK General Practice Research Database [13]. The database, activated in 1990 and storing medical records from the general practitioners, produced a much lower prevalence (from 3.58/1000 in 2004 to 4.09 and 3.90/1000 in 2009 and 2010, respectively), even lower than the median prevalence estimate reported by [7].

In Spain, a two-phase cross-sectional study in the framework of EPINED project was performed in Tarragona (year of study performance not specified), yielding prevalence estimates of 15.5/1000 and 10.0/1000 in the age classes 3–5 years and 10–12 years, respectively [22]. At about the same time, a study was performed using data from the Catalan Public Health Service on children aged 2 to 17 years. The estimated ASD prevalence in 6–10 years old children for 2017 was 11.8/1000, a rather high value for an estimate based on administrative data [23].

### 2.2. Middle-East

Few studies have been performed up to now in Middle East countries, generally yielding prevalence estimates lower than Western Countries.

In Iran, the most updated estimate of ASD prevalence comes from a study that is part of a large-sample national population based epidemiological study concerning psychiatric disorders among Iranian children and adolescents aged 6–18 years [24]. The weighted ASD prevalence estimate for 6–18 years old subjects (computed from data reported in the paper) is approximately 1.60/1000, lower than less recent estimates from United Arab Emirates (2.90/1000 for 0–14 year children) [44], and Israel (4.80/1000 for 1–12 year children) [45].

As already reported in the review by [7], in 2010 a very low countrywide prevalence of 0.14/1000 had been estimated in children aged 0–14 years in the Sultanate of Oman [46]. This prevalence possibly reflected a low capacity of detecting children with ASD more than an actual low proportion of children affected. The lack of biological markers of ASD and the low availability of health services for the diagnosis of and the intervention on children with ASD were examined as factors that may account for the low prevalence [47]. More recently, Al-Mamri et al. performed a multicentre study aimed at updating the estimate of ASD prevalence among Omani children, using data retrieved from the three main centres for the diagnosis of ASD in the Sultanate of Oman in the period December 2011–December 2018 [25]. The new estimate was 2.04/1000 in the overall group of children (0–14 years of age); even if it is almost 15-fold higher than the previous one, it is still very low with respect to most of the estimates worldwide. Within the country, the highest prevalence was observed in Muscat (3.65/1000) with a prevalence in boys 3.4-fold higher than in girls (3.12/1000 vs. 0.91/1000, respectively).

Qatar is a country with a small population (2.7 million) characterized by a very high literacy rate, free and mandatory school attendance, and free healthcare for nationals and residents. A cross-sectional two-phase survey was conducted from 2015 to 2018 to estimate ASD prevalence in children aged 6 to 11 years [26]. The total prevalence (deriving from prevalence of already- and newly-diagnosed cases) was estimated at 11.4/1000, a value much higher than those observed in the other middle-east countries.

In Lebanon, a cross-sectional study was performed in 2014 in nurseries of Beirut and Mount Lebanon, to estimate ASD prevalence in toddlers aged 16–48 months using M-CHAT and a short structured questionnaire developed in the study [27]. Since it was not possible to conduct a follow-up interview to ascertain the M-CHAT results, the proportion of toddlers with a positive result at the M-CHAT was calculated, and corrected by an estimated positive predictive value, yielding a final ASD prevalence of 15.3/1000. This value is quite high and similar to the prevalence estimated in western countries.

### *2.3. Asia*

Qiu et al. [48] have published a systematic review and meta-analysis of studies on prevalence of ASD in South Asia (Sri Lanka, 2009; Bangladesh, 2009 and 2018; India, 2017; Nepal, 2018), East Asia (South Korea, 2011; China, 2011 and 2014), and West Asia (corresponding to the Middle East region: Iran, 2012; Israeli, 2013; Lebanon, 2016). Prevalence estimates show a very large variability across countries, ranging from very low values estimated in Iran, 2012 (0.63/1000) and Bangladesh, 2018 (0.76/1000), to low values estimated in India, 2017 (1.53/1000), China, 2011 (1.77/1000), India, 2017 (2.19/1000), China, 2014 (2.75/1000), Nepal, 2018 (3.42/1000), Israeli, 2013 (4.80/1000). On the contrary, large values were estimated in Bangladesh, 2009 (8.42/1000) and Sri Lanka, 2009 (10.7/1000), and very large values in Lebanon, 2016 (15.3/1000) and South Korea, 2011 (26.4/1000).

The Qiu's review did not include some recent studies performed in China [29–31], in Japan [32], and in India [33]. In China, Yang et al. (2015) [31] performed ASD assessment in 2014 in toddlers (3.8 to 4.8 years of age) who attended mainstream kindergarten in Shenzhen, estimating ASD prevalence at 26.2/1000. Sun et al. (2015) [29] evaluated ASD prevalence in children aged 6 to 11 years from two mainstream schools in Beijing, yielding an estimate of 11.9/1000. In 2019, Sun et al. [30] estimated ASD prevalence in three cities (Jilin, Shenzhen, and Jiamusi) at December 2013, using data from mainstream school only in all the cities and from the whole population in Jilin. Estimates based on mainstream school population were much lower than prevalence estimated in Beijing, ranging from 1.46/1000 in Jilin to 4.23/1000 in Shenzhen, the latter much lower than that estimated from toddlers. On the contrary, the estimate based on the overall population (from mainstream and special schools, private intervention centres, and community not attending school) in Jilin was 10.8/1000, nearer to the estimates from Beijing and from Western countries.

In Japan, a community sample survey was performed to estimate prevalence of neurodevelopmental disorders (NDD) and their co-occurrence in children aged 6–9 years, using questionnaires administered to parents and teachers [32]. The estimated prevalence of ASD, alone or in co-occurrence with other NDD, was 19.0/1000 based on parent's reports, and rose to 93.0/1000 based on teacher's reports. The latter was quite large, much larger than what observed in all other countries. In addition, the agreement rate between parent and teacher estimates was very low, suggesting that teacher's estimate could be largely overestimated and unreliable.

With regard to South Asia, Poovathinal et al. [33] performed a community-based survey in 2011–2012 in Kerala, South India. The study was part of a two-phase epidemiologic survey on chronic diseases performed on the entire regional population. The ASD prevalence in children from 6 to 10 years of age (i.e., the age class showing the highest prevalence) was estimated at 5.05/1000.

Finally, Hoang et al. [37] conducted a two-phase cross-sectional study (screening with M-CHAT and confirmation by clinical assessment) in toddlers from 18 to 30 months of age in Vietnam (Hanoi and Northern provinces). The estimated prevalence was 7.52/1000, obtained as proportion of children confirmed to have ASD on the number of children who underwent ASD assessment. However, the percentage of children undergoing ASD assessment after screening by M-CHAT was 100% in screen-positive children and 2% only in screen-negative ones. In addition, the percentage of ASD confirmation was 52.2% and 0.3% in screen-positive and screen-negative children, respectively. When taking into account the difference in the rate of ASD assessment following M-CHAT screening, and the difference in the rate of ASD confirmation between the two groups of children, the prevalence estimate rose to 10.8/1000, a value much higher than the values from previous studies in the same country, and much more similar to estimates in the Western countries.

### *2.4. Australia & New Zealand*

In Australia, Randall et al. published in 2016 a study performed within the Longitudinal Study of Australian Children (LSAC) framework [38]. Data on the parent-reported ASD diagnoses were collected for children belonging to two different cohorts, recruited in 2004 at birth (B-cohort, years of age 2004) and in kindergarten (K-cohort, years of birth 1999–2000). Data were obtained from two

different waves of the LSAC, referring to children aged 6–7 years in 2010–2011 (for B-cohort, wave 4) and in 2005–06 (for K-cohort, wave 2). Estimated ASD prevalence in 2005–2006 was 14.1/1000, and rose to 25.2/1000 in 2010–2011. Both prevalence estimates were much higher than the previous estimate of 3.92/1000 found by Icasiano et al. [49] in children aged 2–17 years during 2002, living in the Barwon region in Australia. It has to be noted that the estimate by Icasiano et al. refers to children and adolescents in a wider range of age, thus including diagnoses performed in different calendar years that are also affected by different capability of recognizing ASD. Secondly, researchers did not perform ad hoc case ascertainment, basing the estimate of prevalence on formal diagnoses of ASD made prior to data collection. As already reported for the use of data extracted from registries in UK, Italy, and China, prevalence estimates based on existing data are usually lower than estimates coming from ad hoc studies with active ascertainment of cases, and the gap is even larger with respect to estimates based on (often uncontrolled) parent-reported diagnoses (see USA below).

### 2.5. North America

ASD prevalence estimates have been produced in three regions of Canada (Newfoundland and Labrador, NL; Prince Edward Island, PEI; Southeastern Ontario, SO) from 2003 to 2010, using data from the National Epidemiologic Database for the Study of Autism of Canada (NEDSAC) [39]. A general increase of prevalence across years was observed in the three regions, with large differences in prevalence among regions. In children aged 6–9 years, in NL region the prevalence increased from 5.20/1000 in 2003 to 10.8/1000 in 2008. In the PEI region, the prevalence passed from 5.88/1000 in 2003 to 6.13/1000 in 2008, and 9.99/1000 in 2010, and in SO region, from 8.34/1000 in 2003 to 12.4/1000 in 2008 and 16.2/1000 in 2010. Prevalence has been also estimated from2000 to 2015 using data from Quebec Integrated Chronic Disease Surveillance System (QICDSS) [40]. All residents in Quebec for at least one day from January 1, 1996, to March 31, 2015, and aged up to 24 years, were considered eligible for the prevalence study. Physician claims or hospital discharges from 2000 to 2015 reporting a diagnosis of ASD, Rett syndrome or childhood disintegrative disorder at ICD-9 or ICD-10, were used to classify the patient as having ASD. The lifetime prevalence for children aged 1 to 17 years was estimated at 1.50/1000 in 2000–2001, rising up to 12.2/1000 in 2014–2015. In general, a large variability in prevalence rates was observed among sub-areas, with higher prevalence in Montreal metropolitan area and lower in semi-urban and smaller regions.

In the USA, the Centers for the Disease Control and Prevention (CDC) launched in 2000 the Autism and Developmental Disabilities Monitoring (ADDM) Network, with the aim of tracking the number and characteristics of children with ASD in multiple communities in the United States. The ADDM Network is a multisite, multiple-source, record-based surveillance system, providing the most updated and comprehensive estimates of prevalence of ASD and other developmental disabilities in children aged 8 years; this age was chosen because of the peak ASD prevalence observed among elementary-school-aged children. Since 2010, the prevalence is estimated also in children aged 4 years. Prevalence estimates are given from 2000 and every two years (except for 2004); the most recent estimates refer to 2016 [12,41,50–55]. The ADDM Network program uses the systematic screening of databases/registries (related to health, service provision for developmental disabilities, special education) in order to extract information concerning behaviours possibly associated to developmental disorders, building a multi-information record for any child of the specific age class living in the reference geographical area. Information collected in the child's record is then examined to evaluate if the child can be diagnosed with ASD or other developmental disability, based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR). In 2014, 81% of the overall population underwent diagnostic evaluation by both DSM-IV-TR and DSM, Fifth Edition (DSM-5).

Tables 5 and 6 show the USA prevalence estimates in the overall examined populations of children aged 8 and 4 years, respectively, and in subgroups of children based on sex and Ethnicity.

**Table 5.** CDC-ADDM Network ASD prevalence estimates per 1000 children aged 8 years, in the overall group and in sex and ethnicity subgroups, from 2000 to 2016 in USA [12,41,50–55].

| Study Year | 2000 | 2002 | 2006 | 2008 | 2010 | 2012 | 2014 | 2016 |
|---|---|---|---|---|---|---|---|---|
| States (nr.) | 6 | 14 | 11 | 14 | 11 | 11 | 11 | 11 |
| Population | 187,761 | 407,578 | 308,038 | 337,093 | 363,749 | 346,978 | 325,483 | 275,419 |
| Prevalence | | | | | | | | |
| Overall | 6.7 (*1:150*) | 6.6 (1:150) | 9.0 (1:110) | 11.3 (1:88) | 14.7 (1:68) | 14.6 (1:68) | 16.8 (1:59) | 18.5 (1:54) |
| Range | 4.5 WV<br>9.9 NJ | 3.3 AL<br>10.6 NJ | 4.2 FL<br>12.1 AZ, MO | 4.8 AL<br>21.2 UT | 5.7 AL<br>21.9 NJ | 8.2 MD<br>24.6 NJ | 13.1 AR<br>29.3 NJ | 13.1 CO<br>31.4 NJ |
| IQ | | | | | | | | |
| % IQ ≤70 | (40%–62%) | 45% (33%–59%) | 41% (29%–51%) | 38% (13%–54%) | 31% (18%–37%) | 32% (20%–50%) | 31% (27%–39%) | 33% (25%–42%) |
| Sex | | | | | | | | |
| Males | *10.3* | *10.2* | 14.5 | 18.4 | 23.7 (1:42) | 23.6 (1:42) | 26.6 (1:38) | 29.7 (1:34) |
| Females | *2.9* | *2.4* | 3.2 | 4.0 | 5.3 (1:189) | 5.3 (1:189) | 6.6 (1:152) | 6.9 (1:145) |
| M:F | *3.6:1* | *4.2:1* | 4.5:1 | 4.6:1 | 4.5:1 | 4.5:1 | 4.0:1 | 4.3:1 |
| Ethnicity | | | | | | | | |
| White, non-Hispanic | 4.5–11.3 | *7.0* | 9.9 | 12.0 | 15.8 | 15.5 | 17.2 | 18.5 |
| Black, non-Hispanic | 5.3–10.6 | *5.5* | 7.2 | 10.2 | 12.3 | 13.2 | 16.0 | 18.3 |
| Hispanic | *na* | *3.7* | 5.9 | 7.9 | 10.8 | 10.1 | 14.0 | 15.4 |
| Asian/Pacific Islander | *na* | *na* | *na* | *na* | 12.3 | 11.3 | 13.5 | 17.9 |

AL Alabama, AR Arkansas, AZ Arizona, CO Colorado, FL Florida, MD Maryland, MO Missouri, NJ New Jersey, UT Utah, WV West Virginia. Italics indicates data computed from information reported in original papers.

**Table 6.** CDC-ADDM Network ASD prevalence estimates per 1000 children aged 4 years, in the overall group and in sex and ethnicity subgroups, from 2000 to 2016 in USA [1,42].

| Study Year | 2010 | 2012 | 2014 | 2016 |
|---|---|---|---|---|
| States (nr.) | 5 | 5 | 6 | 6 |
| Population | 58,467 | 59,456 | 70,887 | 72,277 |
| Prevalence | | | | |
| Overall | 13.4 (1:73) | 15.3 (1:65) | 17.0 (1:59) | 15.6 (1:64) |
| Range | 8.5 MO–19.7 NJ | 8.1 MO–22.1 NJ | 9.6 MO–28.4 NJ | 8.8 MO–25.3 NJ |
| IQ | | | | |
| % IQ ≤70 | 47.0% | 43.6% | 46.1% | 52.6% |
| Sex | | | | |
| Males | 12.2 MO–31.7 NJ | 12.9 MO–33.6 NJ | 14.2 MO–44.0 NJ | 13.4 MO–38.7 NJ |
| Females | 4.6 MO–7.3 AZ | 3.2 MO–9.9 NJ | 4.3 CO–12.1 NJ | 3.9 MO, NC–11.0 NJ |
| M:F | 2.6–4.4:1 | 3.4–4.7:1 | 3.0–5.2:1 | 3.1–4.9:1 |

AL Alabama, AR Arkansas, AZ Arizona, FL Florida, MD Maryland, MO Missouri, NJ New Jersey, UT Utah, WV West Virginia.

As can be seen, in children aged 8 years the prevalence raised steadily from 6.60/1000 in 2002 to 14.7/1000 in 2010, remained constant from 2010 to 2012, and then raised again, arriving at 16.8/1000 in 2014 and 18.5/1000 in 2016, with an increase of 181% with respect to 2002. ASD prevalence also increased in children aged 4 years, passing from 13.4/1000 in 2010 to 17.0/1000 in 2014, but it decreased to 15.6/1000 in 2016.

In 2010, ASD prevalence was slightly lower in 4-years than in 8-years children (13.4 vs. 14.7/1000; this gap seemed to be bridged in 2014 (17.0 vs. 16.8/1000 in 4-years vs. 8-years children), suggesting an improvement in early diagnosis of ASD, but it appeared again in 2016 (15.6 vs. 18.5/1000 in 4-years vs. 8-years children).

The increase of prevalence from 2008 to 2016 corresponds to a variation in the distribution of ASD-diagnosed children with respect to the intellectual disability (ID; for details see Table 7. The proportion of ASD subjects with moderate ID remains constant across calendar years (24–25%), while the proportion of children with severe ID decreases (from 38 to 31–33%) and that of children without ID increases (from 38 to 42–46%). The prevalence increase with respect to 2008 in children grouped by IQ level suggests the hypothesis that a large part of the increase in the overall prevalence depends on the increase in children without ID, likely due to a greater ability to recognize children with milder forms of ASD (including high-functioning autism and Asperger's syndrome).

In 2018, Xu et al. reported an estimate of ASD prevalence of 24.7/1000 in children and adolescent aged 3–17 years in the 2014–2016 period, based on data from the National Health Interview Survey (NHIS), an annual health survey in the USA [56]. Similar prevalence was obtained by Kogan et al. [57] using data from the National Survey of Children's Health (NSCH) to estimate the national prevalence of parent-reported ASD diagnoses in US children from 3 to 17 years of age in 2016. The estimated value was 25.0/1000 in the overall group of children, and 26.1/1000 in children aged 6-11 years. Both NHIS and NSCH are nationwide surveys (the latter based on a larger sample than the former), and thus potentially representative of the whole country; however, data come from parents, who are asked to report if their targeted child was ever told to have ASD by a doctor or health professional. This introduces possible report biases with not quantifiable effects on the prevalence estimate: for this reason, data are not comparable with those coming from the ADDM Network surveys.

**Table 7.** CDC-ADDM Network ASD prevalence estimates per 1000 children of 8 years of age, in the overall group and in IQ subgroups, from 2008 to 2016 in USA, and percent variation of prevalence with respect to 2008 data (values are computed from original data in [8,12,52,53,55]).

| | 2008 | | 2010 | | 2012 | | 2014 | | 2016 | | 2010 vs. 2008 | 2012 vs. 2008 | 2014 vs. 2008 | 2016 vs. 2008 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | Prev | % | Prev | % | Prev | % | Prev | % | Prev | % | % | % | % |
| Overall | 100 | 11.3 | 100 | 14.7 | 100 | 14.6 | 100 | 16.8 | 100 | 18.5 | +29.5 | +28.8 | +48.4 | +63.7 |
| IQ > 85 | 38 | 4.31 | 46 | 6.75 | 43.9 | 6.41 | 44 | 7.40 | 42.1 | 7.85 | +56.8 | +48.8 | +71.8 | +82.2 |
| IQ 71–85 | 24 | 2.72 | 23 | 3.38 | 24.5 | 3.57 | 25 | 4.20 | 24.1 | 4.51 | +24.1 | +31.4 | +54.6 | +65.7 |
| IQ ≤ 70 | 38 | 4.31 | 31 | 4.55 | 31.6 | 4.61 | 31 | 5.21 | 33.4 | 6.19 | +5.6 | +7.1 | +21.0 | +43.8 |

In Mexico, a survey on children aged 8 years was performed in 2011-2012 in the city of Leon in Guanajuato [42]. Subjects enrolled in the study were students of regular (GSS) or special education (SEMR) schools. Children from GSS underwent a screening phase based on the Social Responsiveness Scale filled in by parents or teachers, and when the score passed the threshold, they were invited to undergo a diagnostic assessment. Based on these data, ASD prevalence was estimated to be 8.70/1000, lower than the prevalence estimated in USA in the same calendar year and quite similar to the USA estimate in 2006.

No prevalence estimates were found for States of Central or South America.

## 3. Factors Potentially Affecting Prevalence

As reported above, Tables 2–4 summarize the studies published worldwide after the review by [15], concerning prevalence estimates in children and adolescents from 1 to 17 years of age ($n$ = 42 studies). Prevalence estimates still vary across and within geographical areas, countries, year of study and source of data used in the study to estimate the prevalence. To detect the contribution of these factors on the variability observed in the prevalence estimates, we performed simple and multiple regression analyses. Specifically, studies were divided into two subgroups according to the age range of subjects (*Agerange*): age group 1 = age range including 7–8 years and/or lower limit of the age range above 5 years ($n$ = 36); age group 2 = upper limit of the age range up to 5 years ($n$ = 6). Prevalence was the dependent variable, while geographical area (*Area*: America, Asia, Australia, Europe, and Middle East), source of data (*Source*: administrative data coming from one or multisource databases, ad hoc study, and report), *Agerange* and year of study performance (*Year*) were the independent variables. As for *Year*, some studies report estimates referring to one specific calendar year ($n$ = 28), in others estimates refer to periods of two ($n$ = 7) or four or more years ($n$ = 3), and others do not specify the calendar year of study performance ($n$ = 4). When more than one year was indicated, we imputed the most recent year of the interval, and when no year was reported we imputed the year before that of study publication. Europe was used as reference level for *Area*, administrative data for *Source*, and age range including 7 and/or 8 years for *Agerange*. Since more studies could be performed in a single country within a geographical area, *Country* (e.g., Italy, France, within Europe area; Oman, Iran within Middle East area, etc.) was considered as clustering factor. The effect of *Year of study* on the prevalence estimate was evaluated in the simple regression. Since, as reported above, *Year* could not be determined with sufficient precision in the 17% of studies, and the effect of *Year* on prevalence estimates in the simple regression analysis was not significant, we did not include this variable in the multiple regression analysis. Variance inflation factors (VIF) were computed for any variable included in the multiple regression model; all VIF values were lower than 5, thus supporting the absence of multicollinearity. The results of regression analyses are presented in Table 8.

The regression analyses present obvious limitations, due to the low number of studies ($n$ = 42), especially in relation to the large number of combinations of area, source of data, and age of children levels ($5 \times 3 \times 2 = 30$ different combinations), making it difficult to disentangle the effects of the different factors. However, we can draw some indication on the potential explanatory factors affecting prevalence estimates.

From the simple regression analyses a significant difference among *Areas* was observed, with Europe showing significantly lower prevalence with respect to Australia ($p < 0.001$). Prevalence estimated based on parents' or teachers' reports was significantly higher than prevalence estimated by administrative data ($p = 0.044$), while neither *Agerange* nor *Year* of study performance significantly affected prevalence estimates.

**Table 8.** Results of simple and multiple regression analyses performed on the selection of studies ($n$ = 42) listed in Tables 2–4.

| Model | | Simple Regression | | | | | | Multiple Regression | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* Studies | Coefficient | 95% CI | | | *p* | $R^2$ | Coefficient | 95% CI | | | *p* | $R^2$ |
| **Area** | | | | | | | | | | | | | |
| *vs* Europe | 14 | | | | | | 0.0302 | | | | | | 0.5314 |
| America | 7 | 2.76 | −3.01 | to | 8.54 | 0321 | | 3.21 | −2.63 | to | 9.05 | 0.254 | |
| Asia | 15 | 2.46 | −12.82 | to | 17.74 | 0.739 | | **−5.58** | **−10.87** | to | **−0.29** | **0.029** | |
| Australia | 2 | **9.45** | **4.78** | to | **14.12** | **<0.001** | | **−41.58** | **−46.87** | to | **−36.29** | **<0.001** | |
| Middle East | 4 | −3.10 | −11.32 | to | 5.13 | 0.435 | | −4.18 | −10.29 | to | 1.93 | 0.156 | |
| **Source** | | | | | | | | | | | | | |
| *vs* Admin + Multisource | 19 | | | | | | 0.3472 | | | | | | |
| Ad hoc assessment | 19 | −3.19 | −8.02 | to | 1.64 | 0.171 | | −0.06 | −5.60 | to | 5.47 | 0.981 | |
| Report | 4 | **27.09** | **0.14** | **to** | **54.05** | **0.044** | | **51.47** | **45.25** | to | **57.68** | **<0.001** | |
| **Agerange** | | | | | | | | | | | | | |
| *vs* Age group 1 [1] | 36 | | | | | | 0.0000 | | | | | | |
| Age group 2 [2] | 6 | −0.18 | −8.48 | to | 8.12 | 0.965 | | **6.26** | **1.47** | to | **11.05** | **0.007** | |

[1] Age group 1 = age range including 7–8 years and/or age range all above 5 years; [2] Age group 2 = age no more than 5 years. Significant effects are highlighted in bold.

Multiple regression model (see Table 8) confirms that studies that use parents' and teachers' report predict higher prevalence in respect with administrative data ($p < 0.001$). On the contrary, since the estimates from Asia were mainly based on ad hoc studies, and those from Australia were both based on reports, when accounting for the source of data prevalence estimates from both areas turned to be significantly lower than those from Europe ($p = 0.029$ and $p < 0.001$ for Asia and Australia, respectively). Finally, estimates in younger children turned out to be significantly higher than estimates in older subjects ($p = 0.007$). Overall, the factors included in the multiple regression model explained about 54% of the variance in prevalence estimates ($R^2 = 0.5314$), notwithstanding that the number of independent variables in the multiple regression model ($n = 7$) was high with respect to the number of studies included ($n = 42$). This suggests the need to investigate other variables, likely related to exposure to different risk factors for autism, in order to explain the observed variability.

## 4. Discussion

The analysis of the literature on ASD prevalence studies published since 2014 confirms a high variability of prevalence estimates worldwide. This variability is still accompanied by methodological differences among the performed studies that concern how cases are detected, which population is involved in, and, to a lesser measure, how cases are defined.

Interestingly, the longitudinal analysis of data across years within the same geographical area confirms the increase of prevalence estimates that has repeatedly drawn scientists' attention in the last twenty years [1]. Studies from Australia [38], Canada [39], Oman [24,25], and USA (see Tables 5 and 6) and some European countries (Sweden, [16]; Italy, [20]) show a substantial increase of ASD prevalence estimates over the years especially at the turn of the 2010. However, the consistency of the increase over countries is masked by the high variability of the prevalence estimates (see Tables 2–4) over the continents, with a range from 0.8/1000 in the North, Sirajganj district of Bangladesh to 93/1000 in Japan.

As previously reported, one of the putative methodological issues contributing to the high variability of ASD prevalence is the source of data from which ASD cases have been detected. From the present analysis, it emerges that the main sources of ASD cases are administrative data (mono or multisource), ad hoc studies, and surveys based on questionnaires. The simple and multiple regression analyses show that the source of data indeed affects the estimate of ASD prevalence. In particular, when ASD cases are detected by teachers' or parents report, prevalence estimated seem to be significantly overestimated. Otherwise, one or more phased population studies appear to produce higher prevalence estimate then studies based on administrative data, but the difference apparently is not significant.

As previously evidenced [58], it is possible that the methodological and qualitative advantages and disadvantages of the use of different sources of data, make it difficult to choose a specific surveillance policy about the count of ASD cases. Population-based designs are considered a high research standard because they are representative of all children in defined populations who meet selected ASD criteria and are evaluated in "natural" community settings, rather than of selected samples attending a particular setting (clinic or educational) or registered in specific research projects. However, the source of identification (teacher; parent; professionals), the lack of blindness of the assessor, the multi-phasing of the study (in relation to the sensitivity of the screening tools and the specificity of the confirmation diagnostic tools) as well as the sample setting (e.g., mainstream vs. special school [29]), and the case definition [59], all represent potential biases that may affect the prevalence estimate obtained by population studies. Otherwise, as above stated, estimates based on administrative classifications have other limitations, due to either difference between states in administrative policies and regulations for the access to the system of recording [60], and/or to socioeconomic disparities or different services availability over the countries [8,9]. Finally, as noted by some scholars, in the survey-based prevalence studies, the formulation of the questionnaire or interview to be administered to parents or teachers can influence the understanding of the questions asked [58] also taking into account educational and/or cultural factors. Furthermore, recall-bias is an intrinsic limitation of this kind of study.

Results of multiple regression also highlight differences in prevalence due to the geographical area where the study is performed, with Europe showing significantly lower prevalence than Australia and Asia. As argued above, this result appears to be at least partially due to the source of data used in the study, but it can indeed be due to several determinants such as socio-cultural [61,62] and socio-economic factors [63], including organisational factors [37,64].

As seen above, factors such as case definition and case-finding procedures, and geographical area, however, scholars reported that they appear to account only for about a 50% of the variability, thus suggesting that additional factors linked to the aetiology of ASD should be considered in explaining variability of ASD prevalence across areas and over time [65–67]. Current literature suggests that several environmental factors could affect brain development and differentiation over perinatal period resulting in neurodevelopmental disorders emerging at different time life. These studies overall focus on dynamic interactions between biological and non-biological risk factors [68]. As for ASD, CHARGE (Childhood Autism Risks from Genetics and the Environment) study is an excellent example of epidemiological study contributing to the understanding of which factors can increase the risk of ASD. Three groups of children have been enrolled in the CHARGE study: children with autism, children with developmental delay but without autism and children from the general population. All of them are evaluated for a broad array of exposures and susceptibilities [69]. Among evidences obtained through the analysis of data collected by the ASD group of children, folate prenatal intake, maternal fever, pesticides exposure, and air pollution, seem to be associated with an ASD risk increase [70]. However, CHARGE study adopts a retrospective case-control approach that ranks in the lower level of the pyramid evidence. Other evidence came from cohort studies that highlighted others possible risk factor such as parental age at birth [71]. Furthermore, some maternal factors (i.e., maternal age, pregnancy and delivery condition, drug intake, maternal autoimmunity, inflammation and chronic stress) are of increasing concern and suggest the need of further studies [72].

In conclusion, multiple and complementary systems are needed to better estimate ASD prevalence and to understand its observed changes. It is necessary to establish either surveillance systems in order to monitor the change of prevalence with time, or guidelines for the performance of ad hoc studies to compare the prevalence across geographical areas. Although the reliability of the prevalence estimates coming from the ADDM Network has been questioned [73], until now this is the only surveillance systems that tracks ASD prevalence over the years and across states, allowing to study factors that possibly give reasons for the observed prevalence increase. Finally, methodological differences across studies could not fully account for the large variation among the prevalence estimates. This suggests the need tostudy other factors, pertaining to the capability to recognize and diagnose ASD and/or to the exposure to genetic and environmental risk factors for ASD, in order to explain the prevalence variation.

**Funding:** This research received no external funding.

**Acknowledgments:** We thank Gemma Calamandrei and Laura Ricceri for critical reading of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Review*

# Systematic Review of Level 1 and Level 2 Screening Tools for Autism Spectrum Disorders in Toddlers

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Received: 28 February 2020; Accepted: 17 March 2020; Published: 19 March 2020

**Abstract:** The present study provides a systematic review of level 1 and level 2 screening tools for the early detection of autism under 24 months of age and an evaluation of the psychometric and measurement properties of their studies. Methods: Seven databases (e.g., Scopus, EBSCOhost Research Database) were screened and experts in the autism spectrum disorders (ASD) field were questioned; Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) guidelines and Consensus-based Standard for the selection of health Measurement INstruments (COSMIN) checklist were applied. Results: the study included 52 papers and 16 measures; most of them were questionnaires, and the Modified-CHecklist for Autism in Toddler (M-CHAT) was the most extensively tested. The measures' strengths (analytical evaluation of methodological quality according to COSMIN) and limitations (in term of Negative Predictive Value, Positive Predictive Value, sensitivity, and specificity) were described; the quality of the studies, assessed with the application of the COSMIN checklist, highlighted the necessity of further validation studies for all the measures. According to COSMIN results, the M-CHAT, First Years Inventory (FYI), and Quantitative-CHecklist for Autism in Toddler (Q-CHAT) seem to be promising measures that may be applied systematically by health professionals in the future.

**Keywords:** autism; level 1 and level 2 screening tools; systematic review; COSMIN; PRISMA

## 1. Introduction

Recently, U.S. data showed that the median age at earliest Autism Spectrum Disorders (ASD; [1]) diagnosis ranged from 28 to 39 months for children aged 4 [2] and is 40 months for children aged 8 [3]. According to these data, a screening procedure during the regular well-baby check-ups was recommended [2,4] with the aim to detect the warning signs of ASD (e.g., precursors of Theory of Mind; [5]). As suggested by several authors [6,7], the process should involve the early screening of warning signs and the subsequent diagnosis made through clinical judgement, in combination with the application of reliable and standardized gold-standard measures (e.g., the Autism Diagnostic Interview-Revised, [8]; the Autism Diagnostic Observative Schedule-2, [9]).

Earlier diagnosis of ASD could lead to earlier intervention for children, which could enhance their adaptation [10–12] or improve their social competence (e.g., emotional expression; see for details [13–15], prevent secondary developmental disturbances [16], and lead to better outcomes [17–19].

Screening measures that are suitable for use in young children (i.e., less than 24 months) are available, and can be classified as either Level 1 or Level 2 instruments [19]. Level 1 screening measures have been developed for the general population (unselected population) to identify children at risk of developmental disorders, including ASD. Level 2 screening tools have been developed to identify children at risk of ASD either because they are already under observation for developmental concerns, or because they failed Level 1 screening, or because they are siblings of children with ASD. The latter, as demonstrated for example by Lauritsen and colleagues [20], have a strong genetic risk. As Robins and Dumont-Mathieu [19] noted, several measures, developed for level 1 or level 2 screening, have been applied to other populations, determining a "hybrid" application of them. The present systematic review focuses on level 1 and level 2 screening measures of ASD, which can be administered to GPs and/or to parents or other professional groups (e.g., nurses, social workers).

In the last few years, eight reviews [21–28] have examined measures for the early detection of risk of ASD. Daniels and colleagues [21] focused on studies investigating approaches aiming at improving the early detection of ASD. This was a systematic review using five databases, although the authors chose to include studies limited to the United States. Garcia-Primo and colleagues [22] and Sappok and colleagues [24] conducted non-systematic reviews considering both measures for the early detection of risk for ASD and for diagnosis. The study by Garcia-Primo and colleagues [22] was limited to measures applied in Europe, published up to 2012, and the search was restricted to two databases (PubMED and PsycINFO). The review by Sappok and colleagues [24] was limited to one database (PubMED) and it considered measures developed for German and English speakers. Zwaigenbaum and colleagues' review [25] was limited to one database (PubMED) and the research strategy included papers published up to 2013. McPheeters and colleagues [23] made a valuable systematic review of the ASD screening tools for children who were referred for developmental disorders other than ASD and were under 36 months old.

Nevertheless, their search strategy included four databases and they considered studies published up to 2000. Marlow and colleagues [26] carried out a systematic review extracted data from four databases and included papers published up to 2017. The meta-analysis by Sánchez-García and colleagues [27] evaluated the accuracy of screening measures according only to their sensitivity, specificity, positive, and negative predictive values (PPV and NPV respectively); furthermore, their electronic search was limited to 5 databases and included paper published up to 2015. Finally, the review by Thabtah and Peebles [28] provided a no systematic review on screening tools administrable from toddlerhood to adulthood, but the authors did not report the search strategy (i.e., databased searched; range of publication years considered) applied and described the tools only in terms of sensitivity and specificity.

Summarizing, most of the above-mentioned reviews are not systematic [22,24,25,28], have limited search strategies to 1–5 databases [22–24,26,27], or focus on a specific geographic area as Europe or USA [21,22,24]. Furthermore, they did not analyze the psychometric and measurement properties of the measures with the exception of Sánchez- García and colleagues [27] meta-analysis which applied the Bayesian Hierarchical Model to evaluate some psychometric properties associated to accuracy. Overall, researchers cannot derive considerations regarding the methodological quality of the studies.

To overcome the limitations of the previous reviews, we provided a systematic search on level 1 and 2 screening tools for ASD and an evaluation of their psychometric properties according to the COSMIN checklist [29,30]. The COSMIN checklist is a 'standardized tool for assessing the methodological quality of studies on measurement properties' [31] developed based on a Delphi study which is a standardized.

The specific research questions were: (RQ1) What are the level 1 and level 2 screening measures to detect early signs of risk of ASD in children under 24 months of age? (RQ2) What are the psychometric properties of the studies of Level 1 and Level 2 measures and what is their quality evaluated applying the COSMIN checklist? (RQ3) Is there one (or more) promising instrument(s) for the early detection of risk of ASD according to COSMIN results?

To give the reader a full and comprehensive view of the characteristics of the Level 1 and Level 2 measures available, and since the COSMIN protocol evaluates the quality of the study, but not the quality of the tool, we collected data on sensitivity, specificity, PPV, and NPV for all the included measures and we provided a discussion about those properties.

## 2. Materials and Methods

The systematic review is based on a published protocol [32], in which the authors reported a comprehensive description of the steps to follow, the methodology, and the process of the review. Furthermore, the authors provided the format of the tables to be used for the main descriptive data of the papers included in the review and the results of the examination of the psychometric properties. The methodology applied was developed based on the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [33] for identifying the papers to be included in the review. An electronic search was conducted using PsychINFO, the Psychology and Behavioral Sciences Collection, Cumulative Index of Nursing and Allied Health Literature, Scopus, the Education Resources Information Center, Google Scholar, and Pubmed (including MEDical Literature Analysis and Retrieval System OnLINE). The keywords applied were: 'early diagnosis or diagnos *', 'ASD screen *', 'ASD detect *', 'ASD or autism or autist *', 'assessment tool', 'surveillance', 'develop * surveillance', 'assess *', 'instrument *', 'measure *', 'psychometric properties', 'standardiz *', 'tool*', and 'validat *'. A secondary hand search was performed to include references and citations from the identified papers. The electronic search was carried out by an author who extracted the records and tabulated the references in an excel file. Two authors independently screened the records to exclude duplicates and to remove papers according to pre-defined inclusion/exclusion criteria. The two authors reported their decisions in two different excel files and they compared their findings record by record. In case of disagreement, a third author arbitrated. Finally, three clinicians and three research experts in ASD, working respectively for the Public Health Service and for Universities respectively, were questioned. Based on the inclusion/exclusion criteria, they did not suggest any other relevant existing measure/study different from those already included in the present review.

Predefined inclusion criteria were: (1) level 1 and level 2 screening measures of ASD for children under 24 months; (2) validation studies, standardization of measures, cross-cultural comparisons, longitudinal, or follow-up studies; (3) published papers in peer- reviewed journals; (4) papers written in English; and 6) a year of publication between 1990 and October 2019. Other reviews on the same topic were examined to extract citations of studies that were eligible for our final list. Furthermore, exclusion criteria were defined as following: (1) measures of the diagnosis of ASD; (2) retrospective studies and systematic reviews; (3) measures of risk detection/diagnosis of others developmental disorders; (4) procedures for the detection of ASD other than questionnaires, interviews and observation procedures (i.e., biological markers, fMRI, blood test); (5) epidemiological studies and guidelines for experts; (6) publications that are not in peer-reviewed journals; (7) papers without the specific aim to evaluate psychometric properties or validity properties of the measures; (8) dissertation thesis or conference papers.

The evaluation of the measures applied the COnsensus-based Standards for the selection of health Measurement INstrument (COSMIN) checklist [29–31]. The COSMIN checklist applies nine boxes identifying the main measurement properties: (A) internal consistency (i.e., the degree to which the items of a questionnaire correlate with each other and evaluate the same concept); (B) reliability (i.e., the ability to measure a construct over time or by different persons); (C) measurement error (i.e., the error of the score not attributed to true changes in the construct); (D) content validity (i.e., the degree to which the items reflect adequately the construct measured); (E) structural validity (i.e., evaluating whether the hypothesized latent factor(s) reaches a good fit of the data); (F) hypothesis testing (i.e., considering whether the construct measured by the questionnaire reaches the expected relations with other variables); (G) cross-cultural validity (i.e., giving information on the generalization properties of the measure when applied in a different cultural context); (H) criterion validity (i.e., the degree to which

the measure correlates with a 'gold-standard' measure); and (I) responsiveness (i.e., evaluating whether the measure predicts a change over time). Each box contains a different number of items (ranging from 5 to 18) evaluating 'design aspects and statistical methods' of a study [31] (p. 651), which require a mandatory assessment to obtain a full appraisal of the properties.

The COSMIN checklist provides a multi-step evaluation. The first step concerns the decision about which measurement properties have been assessed in a target paper among the nine boxes, and it is achieved by applying a binary scale (i.e., present vs. absent) considering the whole paper. For example, if the internal consistency (i.e., box A) is a property evaluated in a paper, then 'present' is attributed to box A for that paper.

The second step refines the evaluation undertaken in step 1. For each box marked as 'present' in step 1, the evaluator works through the questions, assigning to each of them an evaluation on a dichotomous scale ('yes' if the specific properties suggested by the question are present or 'no' if the specific properties suggested by the question are not present).

Finally, in the third step, the score obtained in step 2 is further refined. Every item marked as 'yes' in the previous step is now evaluated on a four-point Likert scale: excellent (+++), good (++), moderate (+), or poor (0).

A final evaluation for each box is obtained by considering the lowest score attributed to that box according to the worst score counts [31] (p. 651) procedure. Therefore, if even only one item in the box obtained a poor score, the measurement property for that box is rated as poor. Two authors independently applied the COSMIN checklist on 20 papers with an inter-rater agreement of Cohen's k = 0.94.

## 3. Results

### *3.1. Overview of the Studies and Measures*

Figure 1 shows the PRISMA diagram.

The electronic search allowed to identify 691 records and a second-hand search added 26 more records. According to the inter-raters decision-making process, during the screening, two authors independently removed 365 duplicates and 300 papers according to the exclusion criteria. The final eligible number of papers included in the systematic review was 52 ([34–85]. The consistency between the two authors who screened these records was high (Cohen's k = 0.89). Sixteen measures were evaluated and classified into 3 categories: observational checklists ($n$ = 4), questionnaires ($n$ = 10), and interviews ($n$ = 2). Table 1 reports the general details of each measure.

Table 2 showed the details of the studies included in the systematic review. Specifically, we reported the measure name, authors and year of the study, the type of the design, population recruited, the application level (1, 2, or "hybrid"), and the diagnostic accuracy properties (i.e., sensitivity, specificity, PPV, NPV).

We found six level 1 measures (i.e., the Checklist for Early Signs of Developmental Disorders; the Early Screening of Autistic Traits Questionnaire; the First Year Inventor; the Joint Attention OBServation; the Screening for Infants with Developmental Deficits and/or Autism; the Young Autism and other developmental disorders CHeckup Tool: 18- month-olds' version) administered to the general population retrieved in 6 longitudinal studies and 3 cross-sectional studies.

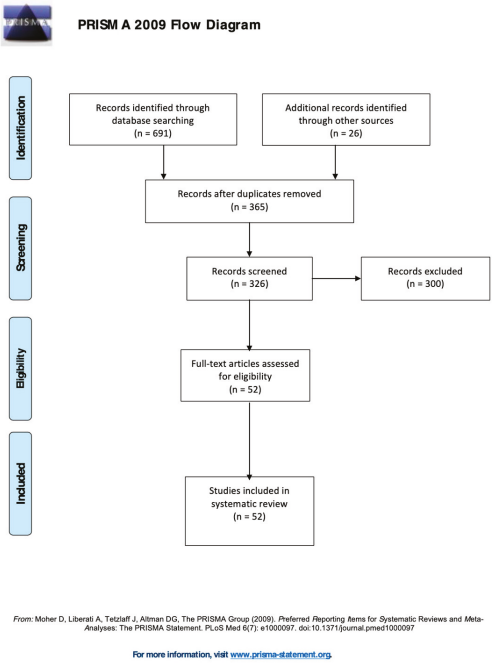


**Figure 1.** PRISMA flow diagram.

### *3.2. Overview of the Studies and Measures*

The search strategy allowed to find four level 2 measures (i.e., the Autism Detection in Early Childhood; the Autism Observation Scale for Infants; the Baby and Infants Screen for Children with aUtIsm Traits; the Parent Observation of Early Markers Scale) that were also retrieved from the systematic search evaluated in eleven studies with a cross-sectional design and in two studies with a longitudinal design. Those measures were administered to two groups of children. The first group consisted of children who were already receiving attention from the local mental health service due to developmental concerns, children suspected of developmental delay, or children qualified for a medical condition that could determine a developmental delay including ASD comorbidity (i.e., epilepsy, hydrocephaly, Down's syndrome, and cerebral palsy). Henceforth this group is identified as Developmental Concerns group (DC). The second group included twins or younger siblings of children with an ASD diagnosis, henceforth defined as Genetic Risk group (GR) because they have high probability to develop ASD [20]. The studies included in level 2 aimed either to: (a) test a screening measure on DC or GR groups; (b) compare DC and GR groups between them; (c) follow DC/GR group until the diagnosis; or, finally, (d) compare children from the general population to DC or GR groups.

Table 2 shows also the details of the six 'hybrid' measures (i.e., the CHecklist for Autism in Toddlers; the Developmental Behavior Checklist: Early Screen; the Modified Checklist for Autism in Toddlers; the Modified Checklist for Autism in Toddlers-Revised with Follow-up; the Quantitative-CHecklist for Autism in Toddlers; the Three-Item Direct Observation Screen) that were developed mainly for level 1 and/or level 2 screening, but they were also administered to clinical populations (i.e., children who had already received a diagnosis of ASD or of another developmental disorder). Those studies aimed either to: (a) apply the measure to a clinical sample, (b) compare samples with different diagnoses (ASD vs. PDD-NOS vs. ODD), or, finally, (c) compare children from the general population with children with an ASD diagnosis. Eleven studies were longitudinal and 19 had cross-sectional design.

**Table 1.** Descriptive details of Level 1 and 2 screening tools include in systematic review.

| Measure Name (Short Name) | Short Description of the Dimension(s) Measured | Admin. Age (Months) | Number of Items | Type of Answer | Admin. Time (Minute) | Admin. Method | Cut-Off | N° of Validation Studies Included |
|---|---|---|---|---|---|---|---|---|
| **Autism Detection in Early Childhood (ADEC)** | Social interaction behaviors and social communication behaviors. | 12–36 | 16 | 3-point Likert scale | 10 | Observational checklist for professionals | 11 | 4 |
| **Autism Observation Scale for Infants (AOSI)** | Social communication behaviors, non- social behaviors, | 6–18 | 18 | 3-point Likert scale | 15-20 | Observational checklist for professionals | n.s. | 3 |
| **Baby and Infants Screen for Children with aUtIsm Traits (BISCUIT)** | Part 1 ASD symptoms; Part 2 comorbid psychopathology; Part 3 behavioral problems. | 17–37 | Part 1: 62; Part 2: 65; Part 3: 17. | 3-point Likert scale | 15 | Parent-interview | Part 1: 17; Part 2: 39; Part 3: 17. | 5 |
| **Checklist for Early Signs of Developmental Disorders** | Language and social functioning. | 3–39 | 25 | Yes/No | Not declared | Parent-reported questionnaire | 2 | 1 |
| **CHecklist for Autism in Toddlers (CHAT)** | Social play, social interest, pretend play, joint-attention, proto-declarative pointing, imitation; B functional play, proto-imperative pointing, motor development, rough and tumble play. | 18 | Part A: 9; Part B: 5 | Yes/No | 15 | Part A: parent-reported questionnaire; Part B: professionals-reported questionnaire | 3 key item | 2 |

**Table 1.** *Cont.*

| Measure Name (Short Name) | Short Description of the Dimension(s) Measured | Admin. Age (Months) | Number of Items | Type of Answer | Admin. Time (Minute) | Admin. Method | Cut-Off | N° of Validation Studies Included |
|---|---|---|---|---|---|---|---|---|
| **Developmental Behavior Checklist: Early Screen (DBC-ES)** | Social, verbal, and non-verbal communication, restricted and repetitive behaviors and interests | 18–48 | 17 | 3-point Likert scale | 5–10 | Parent-reported questionnaire | 11 | 1 |
| **Early Screening of Autistic Traits Questionnaire (ESAT)** | Social- communication skills, stereotyped behaviors, reactions | 14–15 | 14 | Yes/No | 5–10 | Parent-reported questionnaire | 3 | 2 |
| **First Year Inventory (FYI)** | Social communication and sensory regulatory domains. | 12 | 63 | 4 point Likert scale; multiple choice; two open-ended question. | 10 | Parent-reported questionnaire | 30 (95th); 40 (98th); 50 (99th) [73] (Reznick et al., 2007); 22.55 (95th); 28.14 (98th) [39]; 19.2 (96th) [84]. | 3 |
| **Joint Attention OBServation (JA-OBS)** | Joint attention | 20–48 | 5 | Yes/No | 10 | Observational checklist for professionals | 2 | 1 |
| **Modified Checklist for Autism in Toddlers (M-CHAT)** | Social interest, pretend and functional play, joint-attention, proto-declarative pointing, imitation, motor development, rough and tumble play. | 16–30 | 23 | Yes/No | 5–10 | Parent-reported questionnaire | 2 for the critical items (2–7–9–13–14–15) or 3 for the total score | 13 |

**Table 1.** *Cont.*

| Measure Name (Short Name) | Short Description of the Dimension(s) Measured | Admin. Age (Months) | Number of Items | Type of Answer | Admin. Time (Minute) | Admin. Method | Cut-Off | N° of Validation Studies Included |
|---|---|---|---|---|---|---|---|---|
| **Modified Checklist for Autism in Toddlers-Revised with Follow-up (M-CHAT-R/F)** | Social interest, pretend and functional play, joint-attention, proto-declarative pointing, imitation, motor development, rough and tumble play. | 16–30 | 20 | Yes/No | 5–10 | Parent-reported interview | 0–2: low risk<br>3–7: moderate risk<br>8–20: high risk | 7 |
| **Parent Observation of Early Markers Scale (POEMS)** | Social and communicative development, restricted interests, behavioral and emotional problems. | 1–24 | 61 | 4-point Likert Scale | 30 | Parent-reported questionnaire | 70 | 1 |
| **Quantitative CHecklist for Autism in Toddlers (Q- CHAT)** | Social communication, behavior, and language. | 18–24 | 25 | 5-point Likert scale | 5–10 | Parent-reported questionnaire | n.s. | 2 |
| **Screening for Infants with Developmental Deficits and/or Autism (SEEK)** | I: sleep, eating, and parent-child interaction; II: regulation, parent-child interaction, communication, and coordination stability. | 8 | SEEK I: 6; SEEK II: 33 | Yes/No | 3—40 | Parent-reported questionnaire and observational checklist for professionals | n.s. | 1 |

**Table 1.** *Cont.*

| Measure Name (Short Name) | Short Description of the Dimension(s) Measured | Admin. Age (Months) | Number of Items | Type of Answer | Admin. Time (Minute) | Admin. Method | Cut-Off | N° of Validation Studies Included |
|---|---|---|---|---|---|---|---|---|
| **Three-Item Direct Observation Screen (TIDOS)** | Joint attention, eye contact and response to name. | 18–60 | 3 | Yes/no | 15–20 | Observational checklist for professionals | 1 | 1 |
| **Young Autism and other developmental disorders CHeckup Tool: 18-month-olds' version (YACHT-18)** | Motor functions, communication and social interaction, pointing, and language comprehension. | 18 | I: questionnaire (11 items); II: interview (6 questions); II: picture card test. | I: yes/no; III: pass/fail | 10 | Professionals - reported questionnaire; interview with caregivers, child observation | n.s. | 1 |

Note: n.s. = not specified.

**Table 2.** Details of studies included in the systematic review.

| Measure | Author(s) (Year) | Study Design | Population and Subgroups | Application Level (1, 2, or "Hybrid") | Sens. | Spec. | PPV(NPV) |
|---|---|---|---|---|---|---|---|
| ADEC | [34] | Cross sect. | Study 1<br>*N* = 19 ASD<br>*N* = 13 ODD<br>*N* = 29 gen pop.<br>Study 2<br>*N* = 34 PDD<br>*N* = 15 gen. pop.<br>*N* = 5 ODD | hybrid | range: 79%–94% | range: 88%–100% | Study 1: 0.75 (0.90)<br>Study 2: 1 (0.71) |
| | [35] | Long. | *N* = 55 ASD | Hybrid | 100% | 89% | 0.84 (1 *) |
| | [36] | Cross sect. | *N* = 70 ASD<br>*N* = 24 PDD-NOS<br>*N* = 57 ODD<br>*N* = 64 gen. pop. | Hybrid | 100% | range: 74%–90% | 0.84 (1) |
| | [37] | Cross-sect. | *N* = 96 DC | 2 | range: 93%–94% | range: 62%–64% | 0.83 (0.81) |
| AOSI | [38] | Cross sect. | *N* = 101 GR | 2 | N/A | N/A | N/A |
| | [39] | Long. | *N* = 115<br>GR<br>*N* = 73<br>DC | 2 | N/A | N/A | N/A |
| | [40] | Cross sect. | *N* = 54 GR<br>*N* = 50 DC | 2 | N/A | N/A | N/A |

**Table 2.** *Cont.*

| Measure | Author(s) (Year) | Study Design | Population and Subgroups | Application Level (1, 2, or "Hybrid") | Sens. | Spec. | PPV(NPV) |
|---|---|---|---|---|---|---|---|
| **BISCUIT** | [41] | Cross-sect. | Study 1<br>*N* = 957 DC<br>Study 2<br>*N* = 171 ASD<br>*N* = 144 PDD-NOS | 2 | Part 1: 84.7%; Part 2: 84.4%; Part 3: 93.4% | Part 1: 86.4%; Part 2: 83.3%; Part 3: 86.6% | N/A |
| | [42] | Cross-sect. | *N* = 178 ASD<br>*N* = 152 PDD-NOS<br>*N* = 677 gen. pop. | Hybrid | N/A | N/A | N/A |
| | [43] | Cross-sect. | *N* = 276 DC | 2 | N/A | N/A | N/A |
| | [44] | Cross-sect. | Study 1<br>*N* = 405 ASD<br>Study 2<br>*N* = 405 ASD<br>*N* = 882 gen. pop. | Hybrid | N/A | N/A | N/A |
| | [45] | Cross-sect. | *N* = 178 ASD<br>*N* = 152 PDD-NOS<br>*N* = 677 gen. pop. | Hybrid | N/A | N/A | N/A |
| **CESDD** | [46] | Long. | Wave 1<br>*N* = 6.808 gen. pop.<br>Wave 2<br>*N* = 255 at risk<br>Wave 3<br>*N* = 20 ASD<br>*N* = 40 ODD | 1 | 80% | 94% | 0.07 (0.99) |
| **CHAT** | [47] | Cross-sect. | *N* = 50 gen. pop.<br>*N* = 41 GR | 2 | N/A | N/A | N/A |
| | [48] | Long. | Wave 1<br>*N* = 16.000 gen. pop.<br>Wave 2<br>*N* = 10 ASD<br>*N* = 17 ODD<br>*N* = 23 TD | 1 | N/A | N/A | N/A |

**Table 2.** *Cont.*

| Measure | Author(s) (Year) | Study Design | Population and Subgroups | Application Level (1, 2, or "Hybrid") | Sens. | Spec. | PPV(NPV) |
|---|---|---|---|---|---|---|---|
| **DBC-ES** | [49] | Cross-sect. | $N$ = 142 ASD or PDD<br>$N$ = 65 ODD | Hybrid | 83% | 48% | 0.78(0.56) |
| **ESAT** | [50] | Long. | Wave 1<br>$N$ = 31.724 gen. pop Wave 2<br>$N$ = 255 at risk<br>Wave 3<br>$N$ = 18 ASD<br>$N$ = 55 ODD. | 1 | N/A | N/A | N/A |
| | [51] | Long. | Wave 1<br>$N$ = 4.107 gen. pop. Wave 2<br>$N$ = 103 at risk | 1 | N/A | N/A | N/A |
| **FYI** | [52] | Cross-sect. | $N$ = 1300 gen. pop. | 1 | N/A | N/A | N/A |
| | [53] | Long. | Wave 1<br>$N$ = 471 gen. pop. Wave 2<br>$N$ = 17 at risk | 1 | N/A | N/A | N/A |
| | [54] | Long. | Wave 1<br>$N$ = 699 gen. pop. Wave 2<br>$N$ = 9 ASD | 1 | N/A | N/A | N/A |
| **JA-OBS** | [55] | Long. | Wave 1<br>$N$ = 3999<br>Wave 2<br>$N$ = 64 at risk<br>Wave 3<br>$N$ = 48 ASD<br>$N$ = 3 TD<br>$N$ = ODD | 1 | 86% | N/A | 0.90 (N/A) |

**Table 2.** *Cont.*

| Measure | Author(s) (Year) | Study Design | Population and Subgroups | Application Level (1, 2, or "Hybrid") | Sens. | Spec. | PPV(NPV) |
|---|---|---|---|---|---|---|---|
| | [56] | Long. | Wave 1<br>*N* = 1.122 gen. pop.; Wave 2<br>*N* = 171 at risk | 1 | 87% | 99% | 0.80 (0.99) |
| | [57] | Cross-sect. | *N* = 36 ASD<br>*N* = 18 PDD-NOS<br>*N* = 28 ODD | Hybrid | Critic items: 79%; Total score: 88% | Critic items: 38%; Total score: 38% | 0.79(0.28) |
| | [58] | Cross-sect. | *N* = 122 ASD<br>*N* = 106 gen. pop. | Hybrid | 86% | 80% | 0.81(0.93) |
| **M-CHAT** | [59] | Long. | Study 1<br>Wave 1<br>*N* = 2480 gen. pop.; Wave 2<br>*N* = 23 ASD<br>*N* = 63 ODD<br>Study 2:<br>Wave 1<br>*N* = 2055 gen. pop. Wave 2<br>*N* = 6 ASD<br>*N* = ODD | 1 | 100% | 98% | Study 1: 0.35 (1)<br>Study 2: 0.19 (1) |
| | [60] | Cross-sect. | *N* = 24 gen. pop.<br>*N* = 25 DC | 2 | Critic items: 75%; Total score: 65% | Critic items: 89%; Total score: 88% | 0.21(0.98) |
| | [61] | Cross-sect. | *N* = 117 ASD<br>*N* = 339 gen. pop. | Hybrid | N/A | N/A | N/A |
| | [62] | Cross-sect. | *N* = 141 ASD<br>*N* = 102 ODD | Hybrid | range: 70%–97% | range: 38%–99% | N/A |
| | [63] | Cross-sect. | *N* = 447 gen. pop. | 1 | | | |
| | [64] | Cross-sect. | *N* = 552 DC | 2 | range: 70%–97% | range: 38%–99% | N/A |

**Table 2.** *Cont.*

| Measure | Author(s) (Year) | Study Design | Population and Subgroups | Application Level (1, 2, or "Hybrid") | Sens. | Spec. | PPV(NPV) |
|---|---|---|---|---|---|---|---|
| **M-CHAT** | [65] | Long. | Wave 1<br>*N* = 51.853 gen. pop.<br>Wave 2<br>*N* = 173 ASD | 1 | Critic items: 20.8%; total score: 34.1% | Critic items: 97.9%; total score: 92.7% | Critic items: 0.33 (N/A); total score: 0.15 (N/A) |
| | [66] | Cross-sect. | *N* = 2048 gen. pop. | 1 | N/A | N/A | N/A |
| | [67] | Long. | Wave 1<br>*N* = 420 gen. pop.<br>Wave 2<br>*N* = 2 ASD | 1 | N/A | N/A | N/A |
| | [68] | Long. | Wave 1<br>*N* = 1250 DC<br>Wave 2<br>*N* = 18 ASD<br>*N* = 17 ODD<br>*N* = 1 TD | 2 | 67% | With FUI: 99%; Without FUI: 94% | With FUI: 0.60 (0.99) Without FUI: 0.14 (0.99) |
| **M-CHAT-R/F** | [69] | Long. | Study 1<br>*N* = 3309 DC<br>*N* = 484 GR;<br>Study 2<br>Wave 1:<br>*N* = 1.160 DC<br>*N* = 256 = GR<br>Wave 2<br>*N* = 80 ASD<br>*N* = 51 ODD | 2 | N/A | N/A | N/A |
| | [70] | Cross-sect. | *N* = 207 DC | 2 | N/A | N/A | N/A |
| | [71] | Long. | Wave 1<br>*N* = 16.115 gen. pop.<br>Wave 2<br>*N* = 123 ASD<br>*N* = 140 ODD | 1 | 94% | 83% | 0.50 (0.99) |

**Table 2.** *Cont.*

| Measure | Author(s) (Year) | Study Design | Population and Subgroups | Application Level (1, 2, or "Hybrid") | Sens. | Spec. | PPV(NPV) |
|---|---|---|---|---|---|---|---|
| M-CHAT-R/F | [72] | Long. | Wave 1<br>$N$ = 2594 gen. pop.<br>Wave 2<br>$N$ = 253 at risk<br>Wave 3<br>$N$ = 17 ASD | 1 | N/A | N/A | N/A |
| | [73] | Cross-sect. | $N$ = 20 DC<br>$N$ = 128 TD | 2 | N/A | N/A | N/A |
| | [74] | Long. | Wave 1<br>$N$ = 110 gen. pop.<br>Wave 2<br>$N$ = 18 ASD | 1 | 88.9% | 94.6% | 0.76<br>(0.97) |
| | [75] | Long. | Wave 1<br>$N$ = 7928 gen. pop.<br>Wave 2<br>$N$ = 1140 at risk<br>Wave 3<br>$N$ = 72 ASD | 1 | 96% | 86% | 0.69<br>(1) |
| | [76] | Cross-sect. | $N$ = 947 gen. pop. | 1 | 50% | 100% | 100(0.87) |
| POEMS | [77] | Cross-sect. | $N$ = 108 GR | 2 | 74% | 73% | 0.21 (N/A) |
| Q-CHAT | [78] | Cross sect. | $N$ = 779 gen. pop.<br>$N$ = 160 ASD | Hybrid | N/A | N/A | N/A |
| | [79] | Cross-sect. | $N$ = 764 gen. pop. | 1 | N/A | N/A | N/A |
| | [80] | Cross-sect. | $N$ = 139 ASD<br>$N$ = 50 PDD<br>$N$ = 126 TD | 2 | 73–83% | 76–78% | N/A |
| | [81] | Cross-sect. | $N$ = 2400 | 1 | N/A | N/A | N/A |
| | [82] | Cross-sect. | $N$ = 545 | 1 | N/A | N/A | N/A |

**Table 2.** *Cont.*

| Measure | Author(s) (Year) | Study Design | Population and Subgroups | Application Level (1, 2, or "Hybrid") | Sens. | Spec. | PPV(NPV) |
|---|---|---|---|---|---|---|---|
| **SEEK** | [83] | Cross-sect. | $N = 312$ gen. pop. | 1 | N/A | N/A | N/A |
| **TIDOS** | [84] | Cross-sect. | $N = 86$ ASD<br>$N = 76$ ODD<br>$N = 97$ gen. pop. | Hybrid | 95% | 85% | 0.91(0.90) |
| **YACHT-18** | [85] | Cross-sect. | $N = 2.814$ gen. pop. | 1 | 60% | 86.3% | N/A |

Note: Cross-sect. = Cross-sectional study; Long. = longitudinal study; ASD = children with ASD; gen. pop. = general population; ODD = other developmental disorders; PDD = Pervasive Developmental Disorder; PDD-NOS = pervasive developmental disorder—not otherwise specified; TD typically developing children; "hybrid" level of application = level 1 and 2 screening measure applied to other population (e.g., clinical sample); FUI: follow-up interview; Sens = sensitivity; Spec = specificity; PPV(NPV) = Positive Predictive Value (Negative Predictive Value); N/A = not available. * Authors reported PPV and NPV values from [36] study.

The sensitivity, specificity, PPV, and NPV of the measures are extensively reported in the validation studies of the M-CHAT, M-CHAT R/F, and ADEC. For other measures (i.e., CESDD, JA-OBS, POEMS, DBC-ES, and TIDOS) there is only one study, each containing information of the NPV and PPV. All the other measures did not report any positive or negative predictive values. Overall considered, the measures for which the PPVs and NPVs were reported, demonstrated from moderate to high predictive values, although for the M-CHAT results can be considered more stable compared to other measures that need further and deeper exploration of these properties. Quality of assessment of the studies

Table 3 shows the results of the evaluation of each psychometric properties of the studies through the application of the COSMIN checklist. For each box, we reported a summary of the assigned scores.

The quality of assessment revealed a heterogeneous picture. Specifically, 24 studies out of 52 received an evaluation of the internal consistency (Box A) and the scores were fair or poor, with the exception of the studies on FYI and the Q-CHAT, which received excellent scores. The reliability (Box B) was evaluated in 17 studies and the majority of the scores rating from fair to poor. Only studies considering the CHAT and POEMS received respectively an excellent and good evaluation. The measurement error (Box C) was assessed in 5 longitudinal studies and received poor or fair evaluations.

The Box D (i.e., content validity) was evaluated in 9 studies and it received excellent evaluations for studies considering AOSI, BISCUIT, CHAT, FYI, M-CHAT, POEMS, Q-CHAT, SEEK, and TIDOS. Structural validity (Box E) was evaluated in 7 studies, but only 3 received excellent scores regarding two measures (i.e., M-CHAT and Q- CHAT). The Hypothesis testing (Box F) was evaluated for several studies, which received fair or poor scores, whereas those on FYI and the M-CHAT-R/F received good evaluations, and that on M-CHAT was evaluated as excellent. For the studies on JA-OBS, the SEEK, and the YATCH-18 the property was not evaluated.

The cross-cultural validity (Box G) was examined in 11 studies and received fair or poor scores. The box criterion validity (H) was evaluated for all studies, with the exception of the one on Q-CHAT and one on SEEK. This property was rated as excellent or good in four studies for four measures (FYI, M-CHAT, M-CHAT-R/F, and Q-CHAT); whereas for all other studies it was evaluated as fair or poor. Finally, the responsiveness (Box I) was the least-evaluated property with only 3 studies receiving scores from fair to poor.

As Table 3 shows the reasons leading to the attribution of fair and poor scores are above all the missing data and the sample size criteria and the fact that they are evaluated across several measurement properties. These criteria were evaluated by the COSMIN with a conservative approach [86], which will be discussed in the following section.

**Table 3.** Results of the COSMIN evaluation.

| Measures | *Author(s), (Years)* | *Psychometric Properties* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Internal Consistency** | **Reliability** | **Measurement Error** | **Content Validity** | **Structural Validity** | **Hypothesis Testing** | **Cross-Cultural Validity** | **Criterion Validity** | **Responsiveness** |
| **ADEC** | [34] | 0 *unidimensionality, sample* | + *missing item, sample, time interval* | | | | 0 *sample* | 0 *sample* | 0 *sample* | |
| | [35] | | | | | | + *missing item* | | + *missing item* | |
| | [36] | 0 *unidimensionality* | 0 *time interval* | | | + *missing item* | + *missing item* | | + *missing item* | |
| | [37] | 0 *unidimensionality* | | | | | + *missing item, hypothesis* | | + *missing item* | |
| **AOSI** | [38] | | + *sample, missing item* | | +++ | | | | | |
| | [39] | | | | | | + *missing item* | | + *missing item* | |
| | [40] | | | + *missing item* | | | + *missing item* | | + *missing item* | + *missing item* |
| **BISCUIT** | [41] | | | | | | 0 *comparator instrument* | | 0 *statistical methods* | |
| | [42] | | | | | | + *missing item, hypothesis, comparator instrument* | | + *missing item* | |
| | [43] | 0 *unidimensionality* | | | | | | | | |

**Table 3.** *Cont.*

| Measures | Author(s), (Years) | Psychometric Properties | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Internal Consistency | Reliability | Measurement Error | Content Validity | Structural Validity | Hypothesis Testing | Cross-Cultural Validity | Criterion Validity | Responsiveness |
| BISCUIT | [44] | + *missing item* | | | +++ | + *missing item* | + *missing item* | | 0 *statistical method* | |
| | [45] | 0 *unidimensionality* | | | | | + *missing item, hypothesis* | | 0 *no gold standard* | |
| CESDD | [46] | | | | | | + *hypothesis* | | + *missing item* | |
| CHAT | [47] | | +++ | | + ++ | | 0 *comparator instrument* | | 0 *no gold standard* | |
| | [48] | | | | | | + *missing item* | | + *missing item* | |
| DBC-ES | [49] | 0 *unidimensionality* | 0 *only one measurement* | | | | + *missing item, hypothesis* | | + *missing item* | |
| ESAT | [50] | | | *0 time interval* | | | + *missing item, hypothesis* | | + *missing item* | |
| | [51] | | | | | | + *missing item* | | + *missing item* | |
| FYI | [52] | +++ | | | +++ | 0 *statistical method* | ++ | | +++ | |
| | [53] | 0 *statistical method* | | | | | ++ | 0 *statistical method* | ++ | |
| | [54] | | | 0 *time interval, measurement condition* | | | + *sample* | | + *missing item, sample* | + *missing item, statistical method* |

**Table 3.** *Cont.*

| Measures | *Author(s), (Years)* | *Psychometric Properties* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Internal Consistency** | **Reliability** | **Measurement Error** | **Content Validity** | **Structural Validity** | **Hypothesis Testing** | **Cross-Cultural Validity** | **Criterion Validity** | **Responsiveness** |
| **JA-OBS** | [55] | | 0 *only one measurement* | | | | | | + *missing item* | |
| **M-CHAT** | [56] | | | | +++ | . | + *missing item, sample* | | 0 *missing item* | |
| | [57] | 0 *unidimensionality* | | | | | ++ | | ++ | |
| | [58] | | | | | | | + *missing item* | | |
| | [59] | | | | | | + *missing item, hypothesis* | 0 *missing item, translation* | + *missing item* | |
| | [60] | 0 *sample, unidimensionality* | 0 *sample* | | | | 0 *sample, hypothesis* | | 0 *sample, comparator instrument* | |
| | [61] | 0 *unidimensionality* | | | | | + *missing item, hypothesis* | | + *missing item* | |
| | [62] | | | | | | +++ | | 0 *statistical method* | |
| | [63] | 0 *measurement not independent* | | | | | 0 *comparator instrument* | | | |
| | [64] | | 0 *only one measurement* | | | | 0 *comparison instrument* | | | |
| | [65] | | | + *time interval* | | | + *sample, hypothesis* | | 0 *missing item* | |

**Table 3.** *Cont.*

| Measures | *Author(s), (Years)* | *Psychometric Properties* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Internal Consistency** | **Reliability** | **Measurement Error** | **Content Validity** | **Structural Validity** | **Hypothesis Testing** | **Cross-Cultural Validity** | **Criterion Validity** | **Responsiveness** |
| **M-CHAT** | [66] | | | | | +++ | | | | |
| | [67] | | + *missing item* | | | | | 0 *statistical method* | | |
| | [68] | | | | | | 0 *missing item, hypothesis* | | 0 *missing item, sample* | |
| **M-CHAT-R/F** | [69] | 0 *unidimensionality* | | 0 *measurement not indipendent* | | | + *missing item* | | 0 *statistical method* | 0 *statistical method* |
| | [70] | | | | | | | | + *missing item* | |
| | [71] | 0 *unidimensionality* | 0 *administration not similar, statistical method* | | | | + *hypothesis* | | +++ | |
| | [72] | 0 *sample, unidimensionality* | 0 *time interval, statistical method* | | | | ++ | 0 *expertise translator, statistcal method* | 0 *sample* | |
| | [73] | 0 *sample, undimensionality* | 0 *missing, sample, time interval, statistical method* | | | | | 0 *statistical method* | | |
| | [74] | | + *missing item* | | | | 0 *comparator instrument* | + *translation* | 0 *no golden standard* | |

**Table 3.** *Cont.*

| Measures | Author(s), (Years) | Psychometric Properties | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Internal Consistency | Reliability | Measurement Error | Content Validity | Structural Validity | Hypothesis Testing | Cross-Cultural Validity | Criterion Validity | Responsiveness |
| M-CHAT-R/F | [75] | 0 *undimensionality* | 0 *statistical method* | | | | 0 *hypothesis* | 0 *no pilot study, statistical method* | +++ | |
| | [76] | | | | | | | | 0 comparator instrument | |
| POEMS | [77] | 0 *unidimensionality* | ++ | | +++ | | 0 *hypothesis* | | 0 *statistical methods* | |
| Q-CHAT | [78] | +++ | + *measurement condition* | | +++ | | + *hypothesis* | | | |
| | [79] | +++ | 0 *time interval* | | | +++ | + *hypothesis* | | 0 *comparator instrument* | |
| | [80] | +++ | | | | | 0 comparator instrument | | +++ | |
| | [81] | +++ | | | | +++ | 0 comparator instrument | 0 statistical method | | |
| | [82] | + missing item | | | | + missing item | + missing item | 0 no pilot study; statistical method | | |
| SEEK | [83] | | | | +++ | | | | | |

**Table 3.** *Cont.*

| Measures | *Author(s), (Years)* | *Psychometric Properties* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Internal Consistency** | **Reliability** | **Measurement Error** | **Content Validity** | **Structural Validity** | **Hypothesis Testing** | **Cross-Cultural Validity** | **Criterion Validity** | **Responsiveness** |
| **TIDOS** | [84] | | | | +++ | | + *missing item* | | + *missing item* | |
| **YACHT- 18** | [85] | | | | | | | | + *missing item* | |

Note: 4-point scale rating: +++ = excellent, ++ = good, + = fair, 0 = poor. Empty cell = COSMIN rating not evaluated. Ratings fair and poor were explained with the reason(s) in italics leading the evaluation. Specifically, "administration not similar" means the two administration conditions to examine measure property were not similar; "comparator instrument" means that authors did not administered a gold standard measure for ASD to evaluate the criterion validity;"expertise translator" means that the expertise of measure translators was poor or not described by authors; "hypothesis" means that the authors did not formulate the hypothesis a priori; "missing item" means that the authors did not report the percentage and/or the handling method for missing data; "no pilot study" means the translated measure did not pre-tested in a target population; "only one measurement" means the authors did not administered the measure at least two times; "sample" means that the sample size was not adequate;"statistical method" means that authors did not calculated the right parameter(s) for the specifc property;"time interval" means that the time interval between two measurements was not adequate;"translation" means that the back-translation process was not adequately described; "unidimensionality" means that the internal consistency parameter was not calculated for each (sub)scale separately [29,30].

## 4. Discussion

The systematic review identified six level 1 measures and four level 2 measures. Moreover, the present systematic review found that six screening tools were applied to clinical populations. Among the variety of methodologies of the level 1 and level 2 measures, the questionnaire was the most applied due to several inherent advantages. First, questionnaires are normally administered in a very short time, do not require specific knowledge or training, and are much less invasive than observational checklists or interviews. Second, they often do not require specific training on the coding system or the interpretation of the scores. For many questionnaires, the imputation of a final score and the attribution of a meaning to it do not involve any clinical interpretation or specific knowledge of ASD. Nevertheless, questionnaires have several limitations. First, the score depends on the subjectivity of the informants. Since questionnaires are designed for parents, they could under- or overestimate the early signs of risk based on their ability to detect them and to distinguish signs of risk from normal deviation from the developmental trajectories. However, the impact of this limitation could be minimized with longitudinal studies testing and comparing the level 1 and level 2 screening instruments with the gold-standard measures (e.g., Autism Diagnostic Observation Schedule-2, [8]) for the diagnosis of ASD. Another inherent limitation of the questionnaires is social desirability bias in the form of over-reporting desirable behaviors. Future research in this field is needed to develop one or more validity scales, as for other clinical psychological testing procedures (i.e., the MMPI-2; see [87]).

The second aim of the present review was to evaluate the psychometric characteristics of the included measures following the COSMIN checklist. Two main considerations could be drawn by our results, one pertaining to the quantity of the psychometric evaluations and the other to their quality. First, it should be noticed that in the studies included in our systematic review, there are several psychometric properties more frequently evaluated than others. A high number of studies contained data that allowed the evaluation of the internal consistency, reliability, hypothesis testing, and criterion validity; whereas the measurement error, content validity, structural validity, cross-cultural validity, and responsiveness have been evaluated in a low number of studies. The second element to be considered is the quality of the evaluations themselves. Indeed, a high frequency of evaluations of a given property not always corresponds to a high quality of evaluation of that property. For example, the content validity was the property less frequently assessed, compared to the others, but it was rated as excellent for all the studies examined. On the other side, the hypothesis testing was frequently evaluated, but received poor or fair scores. These findings should give an impetus to researchers to design validation studies with a focus on both the quantity of the properties and their quality.

Considered overall, one very common problem for all the studies is the treatment of missing data. Few authors explicitly quantified the missing data in their data set, and very few explained the method that they followed to treat missing data. For studies that aim to identify early signs of risk of ASD, the treatment of the missing data represents a crucial aspect. For this specific case, the imputation of data through statistical procedures risks altering the data structure and the distribution beyond the over-/underestimation of the risk of ASD. Thus, it is quite important that, in the future, researchers explain whether and how they have treated missing data in their sample, especially for the parent-reported measures, for which it is more likely to have items with no answers.

According to the COSMIN evaluation, our findings highlight the necessity of further validation studies for all the measures included in the present review. Longitudinal studies involving general population following a sample over time with the purpose of making a diagnostic evaluation are particularly needed. This will allow for an in-depth study the psychometric properties, to compare the results from different measures, and consequently to increase their criterion validity, and specifically the sensitivity and the specificity through the comparisons with the gold standard measures.

Special consideration had to be drawn regarding the Sensitivity, Specificity, PPV, and NPV of the measures because they are not included in the COSMIN checklist. These properties are extensively reported in the validation studies of the M-CHAT, M-CHAT R/F, and ADEC. For other measures (i.e., CESDD, JA-OBS, POEMS, DBC-ES, and TIDOS) there is only one study each containing information

of these properties (see Table 2 for the specific values). All the other measures did not report any positive or negative predictive values. Overall considered, the measures for which the Sensitivity, Specificity, PPVs and NPVs were reported, demonstrated from moderate to high predictive values (see also [27]), although for the M-CHAT results can be considered more stable compared to other measures that need further and deeper exploration of these properties.

The third and final research question aimed at the identification of one (or more) promising instrument(s) for the assessment of early signs of risk of ASD according to the COSMIN evaluations of the studies. We consider the questionnaires such as the FYI, the M-CHAT, and the Q-CHAT as promising screening measures because, according to the COSMIN evaluation, they have high number of psychometric properties evaluated and high methodological quality attributed to them. Although we found these measures promising, none of them can be currently considered as the gold standard in the early detection of risk of ASD and further development in this field is desirable. For example, future studies should improve sensitivity, specificity, NPV, and PPV properties of those measures since they are not considered at all for the FYI and they are barely considered for M-CHAT and Q-CHAT, as also suggested by [27].

On the contrary, the interviews and the observational checklists have both low number of validation studies (with the exception of the M-CHAT-R/F) and low methodological quality attributed to them. Further research should be developed on these methods of evaluation focusing on their psychometric properties, as it may be useful for health professionals to have a range of tools available for ASD risk detection that allows an in-depth analysis.

The present systematic review has several limitations. First, the COSMIN checklist is a standardized protocol for the assessment of the methodological quality of a study and not of the instrument itself. However, as suggested by others [see 86] the evaluation of the methodological quality of a study is the first step to determining whether its results are reliable and trustworthy. In other words, evaluating the methodological quality of a study allows to discover risk of bias in the results. Thus, the assessment of the quality of the study is directly related to the assessment of the measure administered in that study. Moreover, one of our inclusion criteria considered all the "validation studies, standardization of measures, cross-cultural comparisons, longitudinal, or follow-up studies", which are studies evaluating measurement and validity properties of a screening measure. Therefore, we applied the COSMIN checklist to evaluate measurement properties of studies that, in turn, evaluate the measurement properties of the screening measures. Thus, the evaluation of the properties of a study, in this case, is a proxy of the evaluation of the measure validated in that study.

Second, the worse score counts policy of the COSMIN could lead to a negatively biased view of the measure. In this vein, the COSMIN itself explains that every item of its evaluation represents an important part of the overall assessment, so a poor rating for any item should be considered as a serious flaw. Furthermore, we would like to focus on the COSMIN evaluation of the sample size. According to [31], the sample size is evaluated as excellent when it is $\geq 100$, as good when it ranges 50-99, fair when it ranges 30-49 is fair, and poor when it is $< 30$. This categorization is a good criterion when applied to the general population, while when risk and/or clinical groups are considered, the COSMIN sample evaluation should be carefully considered according to the prevalence rate of ASD. According to this premise, recently, the researcher who developed the COSMIN protocol reformulated the evaluation of the sample size (see [86]).

Third, the Sensitivity, Specificity, Positive Predictive Value (PPV) and the Negative Predictive Value (NPV) are not evaluated in the COSMIN checklist. Within the context of screening measures for ASD, it is important that professionals are confident when using a given tool. In this field, the predictive values provide valuable information on the probability of a tool to identify that people with high scores indeed have high risk (PPV) and, vice versa, that people with low score have low risk (NPV). To avoid the omission of such important information, we extracted values of the NPVs and PPVs from the studies, we reported them in Table 2 and we discussed the evidence.

Finally, like every systematic review, the definition of inclusion criteria could have limited the electronic search, and we could have omitted several studies.

The present systematic review has two main strengths. First, the review provides an updated and complete overview of the current level 1 and level 2 screening measures for ASD. Second, our findings provide researchers and clinicians (i.e., pediatricians, GP, psychologist) the analytical knowledge on psychometric properties of the measures through the evaluation of the methodological quality of their validation studies. The outcomes of the systematic search and the results of the evaluation of the psychometric properties, through the application of the COSMIN criteria, may guide researchers and clinicians in their selection of one (or more) instrument(s), according to their specific purposes. A critical and reasoned choice of a measure combined with the good communication between clinical and patients [88] could allow for defining systematic screening procedure on general population. This is the first step for early identification of risk of ASD, which, in turn, may lead to a timely diagnosis and ultimately to better outcomes for children [10,17,18] and families [89].

**Author Contributions:** Conceptualization, F.L. and S.P.; methodology, S.P. and A.L.; software, A.L.; investigation, A.L.; data curation, S.P. and A.L.; writing—original draft preparation, S.P.; writing—review and editing, S.P. and A.L.; supervision, F.L.; project administration, F.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Review*

# Pre-School Teachers' Knowledge, Belief, Identification Skills, and Self-Efficacy in Identifying Autism Spectrum Disorder (ASD): A Conceptual Framework to Identify Children with ASD

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Received: 29 February 2020; Accepted: 8 March 2020; Published: 13 March 2020

**Abstract:** Recently, the identification and detection of children with autism spectrum disorder (ASD) has become an essential issue under ASD intervention services. The high percentage of ASD among children requires preschool teachers to recognizse children's abnormal development and identify them at an early stage, followed by referral to specialists. Therefore, this identification calls for a specific ability among preschool teachers, identified as knowledge, belief, identification skills, and self-efficacy (KBISSE). This conceptual framework aims to utilize the current literature to present a discussion on preschool teachers' KBISSE in identifying children with ASD and making decisions to refer children suspected with ASD to specialists. The conceptual framework is discussed based on social cognitive theory (SCT) and the health belief model (HBM). The conceptual framework emphasizes the need for preschool teachers to be educated in ASD via an educational module that could increase teachers' self-efficacy in identifying children with ASD. Besides, knowledge in ASD, belief in ASD, and identification skills are also necessary variables for building the educational module. The educational module is useful for guiding future research on preschool teachers' identification of children with any disability, one of which is ASD, and subsequent specialist referral at an early stage.

**Keywords:** preschool teachers; self-efficacy; knowledge; belief; skills; identify; autism spectrum disorder (ASD)

## 1. Introduction

Recently, professional concern towards children with behavioral difficulties is now viewed as an integral part of the teacher's role [1]. One of these behavioral difficulties is an autism spectrum disorder (ASD).

ASD is considered the most common multifactorial disorder affecting children today [2–4]. Currently, the high percentage of ASD among children requires preschool teachers to recognize children's abnormal development and identify them at an early stage followed by referral to specialists [5–7]. A preschool teacher has a high chance of detecting this type of disorder among his or her students and could identify the student's situation to refer them for appropriate assessment towards obtaining early intervention services [8]. However, preschool teachers might assume many obstacles in identifying and referring their students for assessment [8–10].

These obstacles could include personal characteristics of preschool teachers such as the preschool teachers' beliefs, attitudes, feelings, skills, and perceptions of children with ASD, and the preschool teachers' knowledge in managing ASD. So, the lack of knowledge coupled with incorrect beliefs

toward ASD could lead to preschool teachers having a weak self-efficacy in identifying ASD in children and less confidence in voicing their concern to the children's parents and then referring them for early intervention.

Self-efficacy is one of the teachers' characteristics that reliably affects their teaching practices, classroom teaching, and communication with children [11]. According to Bandura [12], self-efficacy can be defined as the preschool teacher's ability to take action and handle children with challenging behavioral problems like ASD (p. 270). One study suggested that teachers who believed in their ability to handle behavioral issues like ASD would put in the effort to create change for the affected children, and vice versa [13], because preschool teachers have the most important role in identifying children with ASD and referring them for clinical intervention at an early stage [8].

An important party in the early identification of children with ASD is preschool teachers, as they are considered reliable resources for intervention issues [14–16] due to their role in dealing with parents to point them towards intervention services. Furthermore, preschool teachers deal with children daily and have been educated in child development [17,18]. Due to these specific characteristics, preschool teachers should have the best qualities to identify children who do not exhibit signs of normal development at an early age [9].

Other researchers have found that a shortage of preschool teachers' knowledge and skills in handling behavioral difficulties was the main factor affecting their referral ability [19]. Another study found several teachers with a shortage of knowledge and skills in handling preschoolers with challenging behavior and recommended them for training [20].

In other words, the preschool teachers' beliefs, understanding, knowledge, and skills related to preschoolers' challenging behaviors might impact their identification of ASD and referral decisions later [14–16,19].

This study attempts to explore how preschool teachers can acquire the ability to identify children with autism and refer those suspected with ASD to specialists while working with the children's parents at the same time. Furthermore, the present research studies the effect of preschool teachers' ASD knowledge and skills in identifying children with ASD. It aims to correct the misbelief in society regarding ASD, and to increase preschool teachers' self-efficacy in recognizing the symptoms of ASD, the factors to increase their confidence to voice their concerns to parents, and boost their willingness to refer children for screenings and other services. Therefore, the main purpose of this study is to conduct a comprehensive discussion of the literature and existing theories to build a conceptual framework that would prepare preschool teachers to identify children with ASD and make the decision to refer children suspected with ASD to intervention services. Figure 1 shows the problem statement identified in this study.

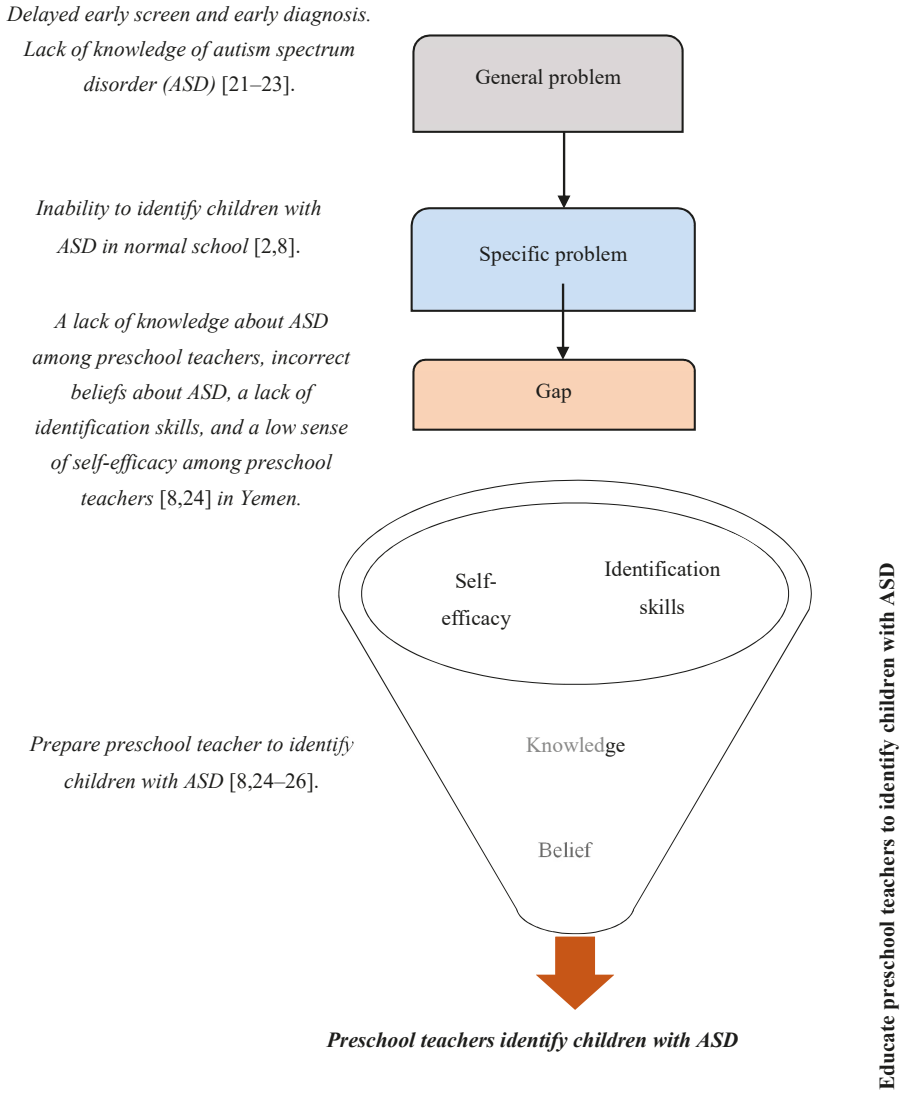


**Figure 1.** The research problem statement [2,8,21–26].

### 1.1. Autism Spectrum Disorcer (ASD) Knowledge among Preschool Teachers

ASD knowledge among preschool teachers refers to general information on ASD, symptoms of ASD, ASD treatment, and the etiology of ASD among preschool children. Thus, the teachers' knowledge affects their identification of children with ASD and their referral decisions [14–16,19].

The preschool teachers' lack of knowledge in screening and identifying children with ASD becomes one of the most significant barriers in the intervention issues of ASD [21]. As defined by several studies around the world, there is a lack of ASD knowledge among teachers [22,23,27] particularly regarding the early signs of ASD [28–30].

The *Diagnostic and Statistical Manual of Mental Disorders* (*DSM*) 2013, has classified or categorised many disorders under ASD such as Asperger disorder and pervasive developmental disorder.

Furthermore, they have determined just two main criteria for ASD diagnosis. One is difficulties in social communication and the other is restrictive and repetitive patterns of behaviour. These new classifications and criteria shifted the way people think about autism and enhanced the development of many instruments related to diagnosis tools of ASD, people's knowledge about ASD, and their ability to identify ASD [31]. However, in developing countries like Yemen, it is a big challenge to use some of these ASD diagnosis tools for several reasons [31,32]. First, using these tools needs well-trained clinicians and experts to ensure the accurateness of the diagnosis [32]. Second, the lack of centers and experts who work with autism. Moreover, Yemeni preschool teachers don't have in-depth knowledge about ASD and are not trained to deal with special needs students in general [33,34]. Therefore, they might not be qualified to use ASD diagnosis tools to avoid wrong interpretation of the outcomes of these tools. Besides, studies have indicated that only 8% of assessments of ASD among children use a formal measure while most use informal assessments [32,33].

Hence, recent studies are now insisting on educating teachers about the early signs of ASD to enable them to identify early symptoms of ASD and to refer the children to professional assistance in the first stage of childhood [22]. Preschool teachers with a low level of ASD knowledge require urgent training [35,36].

Past studies on teachers found that an absence of knowledge and skills in handling early childhood students with challenging behaviors is an obstacle in the detection and intervention of ASD. The literature shows that preschool teachers have been questioned about the factors they observed as impacting their referral decision of children with behavioral difficulties—to which they expressed a lack of knowledge as one of the most crucial factors affecting this ability [9]. Moreover, the teachers rated the identification of ASD and the referral of suspected children with ASD as more important than any other issue [20]. (For more details of the literature review in the knowledge of ASD, see Table 1).

**Table 1.** Summary studies of knowledge about autism spectrum disorder (ASD).

| Authors\Years | Objective | Sample | Instrument | Result | Conclusion |
|---|---|---|---|---|---|
| Taresh et al., 2020 [33] | The current study aimed to figure out what is the pre-school teachers' knowledge about ASD. Besides, this study attempted to find out if there are any significant differences in preschool teachers' knowledge about ASD in relation to their education level and teaching experience. | A total of 300 preschool teachers from various region schools in Taiz City in Yemen. | Questionnaire to determine their level of knowledge about autism. | The results indicated that preschool teachers had a lower level of knowledge about the disorder. The findings also showed significant differences in the teachers' knowledge about autism, depending on their education level and teaching experience. | In conclusion, Yemeni preschool teachers need more education and training in autism spectrum disorder. |
| (Hof M., 2020) [1] | The study evaluated the knowledge of ASD and stigmatizing attitudes. | Physicians at Dutch Youth and Family Centers (YFC). | Questionnaire. | The physicians had positive attitudes toward mental illness but they had higher levels of stigmatizing attitudes than other Western healthcare professionals. Their levels were considerably lower than in non-Western professionals. We found no relations between ASD knowledge, stigmatizing attitudes, and demographic variables. | In conclusion, ASD knowledge and stigmatizing attitudes toward mental illness in Dutch YFC physicians require attention. |
| (Badam, 2019) [37] | The current study aimed to determine and compare the awareness level of ASD among participants including medical and non-medical professionals. | The participants were nursing trainees versus teachers(200 participants), 100 nursing trainees and 100 teachers. | A questionnaire comprising of 19 questions categorized in five sections on various communication disorders. | The current study found a higher level of awareness of communication disorders amongst the medical professional and non-medical group. | It concluded that there is a need to spread constant awareness by awareness campaigns about ASD. |
| (Sasson, 2018) [35] | To examine the effect of an early screening training on pediatric Physical Therapists (PTs): (1) Knowledge of autism spectrum disorder (ASD), (2) clinical self-efficacy, and (3) identification of markers. | Twenty-six pediatric PTs participated in a two-day 'Early ASD Screening' workshop. | Questionnaire in both ASD knowledge and self-efficacy, and video case study | The result confirmed that there is an increase in PTs' knowledge and self-efficacy after the ASD workshop, as compared to before the workshop, and the PTs' ability to identify the early signs of ASD is greater than before the workshop. | It concluded that the workshop was useful to increase the level of knowledge and self-efficacy among PTs. |

**Table 1.** *Cont.*

| Authors\Years | Objective | Sample | Instrument | Result | Conclusion |
|---|---|---|---|---|---|
| (Rakap et al., 2018) [38] | They examined the teachers' knowledge and perceptions of ASD. | A total of 478 general education teachers in Turkish schools. | Questionnaire in knowledge and self-efficacy. | The teachers have a low level of knowledge and misconceptions about ASD. | The results confirmed that there is an urgent need to develop module or certification programs to train teachers to understand this kind of disorder and to work with ASD childrens' implications for future research. |
| (Sanz-Cervera et al., 2017) [39] | This study aimed to examine and compare the pre-service teachers' knowledge, misconceptions, and gaps about autism in their first and final year at university. | Pre-service teachers, n = 866. | Questionnaire. | The finding showed that fourth-year students had higher levels of knowledge and fewer gaps than the first-year students, although they also had more misconceptions. Special education specialists obtained significantly more knowledge and fewer misunderstandings than the general education pre-service teachers. However, specific training and experience had a significant influence on the knowledge and gaps, but it had no impact on the number of misconceptions. | These results suggest that university preparation in autism spectrum disorder (ASD) might not adequately train all future teachers. |
| (Heys et al., 2017) [40] | Examined parents' and professionals' understanding of autism in one low-income country, Nepal. | Parents of autistic and non-autistic children and education and health professionals, n = 106. | Semi-structured interviews. | The result showed there was a lack of knowledge among the participants. This study shows the striking lack of awareness of autism by parents and professionals alike in one low-income country. | |
| (Al-Sharbati et al., 2015) [24] | Studied children with special needs such as those with an autism spectrum disorder. | A total of 164 teachers were randomly selected through five schools. | A cross-sectional study to gauge the knowledge and attitude of mainstream school teachers towards ASD in an urban region in Oman. | The results confirmed that misconceptions about autism spectrum disorder were found to be common among mainstream teachers in Oman. | |

**Table 1.** *Cont.*

| Authors\Years | Objective | Sample | Instrument | Result | Conclusion |
|---|---|---|---|---|---|
| (Shamsudin, Rahman Abdul, 2014) [41] | The study aimed to provide preliminary insight into the awareness of children with autism among the general public in Malaysia. | The general public in Malaysia, n = 250. | Questionnaire. | This study found that, although there are many Malaysians familiar with the term autism, most of them still do not really understand the characteristics of children with the disorder. | |
| (Neik et al., 2014) [42] | The study highlighted the current prevalence, diagnosis, treatment, and research on autism spectrum disorders (ASD) in Singapore and Malaysia. | A review paper from a different database. | ———————— | Based on database searches, it was found that awareness about autism among the lay and professional public is higher in Singapore compared to Malaysia. | |
| (Haimour and Obaidat 2013) [43] | This study endeavored to find out what school teachers knew about autism. | A total of 391 general and individual education teachers in Jeddah in Saudi Arabia. | Completed a study tool (autism knowledge questionnaire) to measure level of knowledge about autism. | It was found that among the participants, the knowledge about autism disorder ranged from satisfactory to almost weak. | |

According to Bandura [44], even if the individuals have the knowledge to complete a task, it does not guarantee that they would actually implement the task. The research mentioned the central role of self-efficacy in gaining the knowledge to be applied in one's work and what one actually does. Self-efficacy is usually deliberately discussed with knowledge because, as some researchers have figured out, base-level knowledge may be needed to be able to perform some actions [45]. This relationship between knowledge and self-efficacy directly influences the individual's performance or capabilities in an instructive system. As teachers, self-efficacy tends to be the most crucial role in impacting their confidence in implementing their knowledge in several situations [46], as cited in Soto et al. [47].

In contrast, other studies found that the strongest factor contributing to high self-efficacy was "confidence in knowledge" (e.g., obtained via teaching experience, teacher training, professional development, and personal knowledge). When asking 84 pre-service teachers and 156 experienced teachers to name the factors that affect increased self-efficacy [2], the most frequently cited was confidence in knowledge for experienced teachers; but the most popular reason for high self-efficacy among teachers was cited to be personal qualities (e.g., concerned attitude, motivation, positive position, and the ability to get along with people) [48]. That is, both groups cited confidence in knowledge, teaching experience, and managing the class as the factors that most influenced self-efficacy. Therefore, knowledge is essential in gaining self-efficacy. Besides, this result mirrors Bandura's [44,49] view that recognized knowledge and experience as a form of behavioral capability and the main cause of self-efficacy.

According to the above association between knowledge and self-efficacy, another crucial variable to consider is the assessment of the preschool teachers' level of knowledge in dealing with abnormal development such as ASD. Specifically, several works have indicated that, generally, teachers do not have the capabilities necessary to deal with special-needs children [50]. Therefore, some noted that although there is a larger than average need for special-needs education services such as a head-start program, there are insufficient resources (such as trained personnel) to address these needs [16].

### *1.2. Beliefs about ASD among Pre-School Teachers*

Beliefs "play a critical role in defining behavior and organizing knowledge and information" (p. 328) [51]. The health belief model (HBM) suggests that a person's belief regarding a personal threat of an illness or disease, together with a person's belief regarding the effectiveness of the recommended health behavior or action, will predict the likelihood that the person will adopt the behavior.

Other investigations found that there are valid causes for realizing the educational beliefs of pre-service teachers as essential to the teachers' education module—because these beliefs majorly impact the pre-service teachers' knowledge achievement, their clarification of knowledge and course content, teaching behavior, task description and collection, and "comprehension monitoring" [51] (p. 313–328) [52]. Pajares [53] defined that beliefs reflect some type of understood knowledge. Also, the author determined that some scholars view beliefs as a portion of knowledge, while others view beliefs to be a portion of conception. Furthermore, the author declared that these beliefs could shape "one part of an individual's meta-cognition" (p. 2). One study proposed an interesting assumption that "beliefs influence what teachers say outside the classroom, but their behavior in the classroom is a result of beliefs measured and filtered by experience. Also, their knowledge represents their efforts to make sense of their experience" (p. 312) [51].

The belief regarding ASD refers to teachers' emotional state and concerns about the children's behavior that influences their ability to identify, voice their concern to the children's parents, and then refer them to specialists and time their decisions.

This belief is divided into two types: religious and personal beliefs. Teachers' belief reflects the difficulty in voicing or discussing their concerns about their perception of the difficult behavior of the child to his or her parents, even addressing or referring the preschooler as having challenging behavior based on the misbelief surrounding ASD among teachers and parents [9]. Furthermore, other

concerns related to a person's beliefs can affect the central role of how individuals understand and explain incapacity and children with ASD [54].

Studies have indicated that feelings may also impact teachers' identification and referral decisions. For example, teachers may view it as easier to talk with parents regarding their children's speech and language problem rather than discussing the possibility of a mental disorder, as the former is less stigmatizing than the latter. Fantuzzo et al. [14] agreed that identifying children with speech and language problems even when there is no speech or language problem present may be done as a means to "avoid the negative consequences of a more stigmatizing and continuing label" (p. 478). This view suggests that there may be biases or fears related to mental health or illness in general and in early childhood in specific. Besides, Fantuzzo et al. [9] proposed that teachers might experience stress when dealing with a child with behavioral problems such as hyperactivity, causing a social disturbance, consideration problems, and non-subjugation. Making a referral to speech and language services, because they are more accessible and less stigmatizing, may provide teachers with more direct help than what may be obtained by waiting for mental health services. While all these suggestions appear sensible, an additional study on teachers' beliefs that cause them to harbour bias against making referrals because of feelings and behavioral problems has yet to be conducted.

In this study, preschool teachers' belief was divided into two: religious and general. Religious belief refers to religion and spiritual traditions that are often associated with health practices observed in cultures around the world [55]. Teachers' beliefs affect their ability in making referral decisions, as Muslim families believe that God puts an autistic child under their care not only because of fate or reincarnation but also because God wants to assess the families to see if they could take care of the child [54]. Religious implications on the beliefs about children with developmental problems are not only limited to Muslims believers alone [3] but are also inclusive of other religious groups. For example, as reported in a previous study, 55% of Latina mothers believed that their autistic child is a sign of God's existence [44], such as, Latina mothers believe that ASD is blessings or gifts from God. [45]. Latin Americans have the option of opting for non-traditional treatments, and numerous Hindu parents of children with "mental disorders" believe that God has given them the child as a response to sins committed in their previous lives [47]; also, Americans use traditional treatments and professional services with behavioral health [46], and ultra-orthodox Jewish families often change community dynamics by receiving medical advice from a Rabbi [48].

Meanwhile, general belief refers to culture and personal belief. This belief is related to society's common belief system and serves as an explanatory model for disorders such as ASD. On the negative side, culture makes people perceive ASD as a stigma. The stigma surrounding autism has resulted in discrimination not only against autistic children but also their families [56]. Moreover, most children with ASD have gone unidentified due to the fear of social opinion among the parents and children [55]. Furthermore, the *Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition (*DSM-5*) considers culture as the main standard for judging whether or not certain behavior is abnormal. Several studies have focused on the role of culture, society, race, and the types of social relationship factors in determining beliefs regarding psychological disorders.

Moreover, personal belief is related to preschool teachers' diagnosis of ASD causes and symptoms, or general information that reflects their attitude or thinking [51]. Besides, this belief often differs from groups of people such as those with low education level, or some individuals with unique characteristics, like those of the Arab community. For example, the common Arabic word for autism describes individuals with a behavioral, mental, physical, and emotional disability, but often the term is translated to 'introvert' or 'withdrawn' in English or (التوحد) in Arabic. Hence why many individuals may incorrectly describe the nature of ASD as introversion [57]. Furthermore, the literal translation of both Chinese terms for autism (GuduZheng or ZibiZheng) is similar to 'loneliness' or 'introvert disease,' which implies a more psychological etiology [58]. (For more details about the literature review in beliefs of ASD, see Table 2).

**Table 2.** Summary studies of beliefs about ASD.

| Authors\years | Objective | Sample | Instrument | Result | Conclusion |
|---|---|---|---|---|---|
| (Samadi, 2020) [59] | Identification, description, and treatment of ASD in Iran. | A total of 43 Parents of children with ASD (27 mothers and 16 fathers). | Questionnaire. | The study result found Iranian parents had their special justification regarding their experience with ASD. Early child development and interventions must be understood within the cultural context. | The study suggested that the culturally informed researcher on ASD is vital to boost awareness of the importance of understanding parental concerns and their need for educational and psychological services in countries in which autism is less known, misdiagnosed, undiagnosed, or even stigmatized. |
| (Sheely, 2020) [60] | The aims of this study were to examine the context of the Indonesian government's intention to develop an inclusive education system. | A total of 136 from teachers and educational therapists. | Questionnaire. | The data suggest that having access to information about autism in the Bahasa Indonesia language plays a role in educators' beliefs about the stigmatization of teachers and parents of autistic children. Teachers' epistemological beliefs were found to be linked to their beliefs in inclusive education. | |
| (Warstadt M., 2020) [61] | This study aimed to assess the public perceptions about autism spectrum disorder (ASD) among United States citizens by using Mechanical Turk. | The participants answered a survey about beliefs regarding causes, treatments, and general information of ASD. | Survey tool by online recruitment. | The results confirmed that participants who had a child with ASD were more likely to attribute ASD to external causes than those without connections to ASD. | The study' result will support awareness campaigns. |
| (Stronach et al., 2019) [62] | The study aimed to explore autism understanding and stigma among university students, and general community members recruited at a state fair. | The result was that all the responses of ASD-Q fell within the adequate knowledge range, indicating relatively high levels of autism knowledge and low levels of stigma. | ASD-Q questionnaire. | The results of this study recommend the need for a continuous investigation into tools that indicate autism understanding and stigma. | |

**Table 2.** *Cont.*

| Authors\years | Objective | Sample | Instrument | Result | Conclusion |
|---|---|---|---|---|---|
| (Jegatheesan, et al., 2010) [54] | The study aimed to investigate the beliefs about autism among three multilingual immigrant South Asian Muslim families who have children with autism. | Parents. | Interviews and conversations recorded during 17 months. | The study's results indicate that families have viewed that their primary purpose is to raise their child to incorporate them into daily social life, linguistic, and religious practices at home and in the community. On other words, Muslim families understand that the task of raising a child with autism in religious terms is the proper way to educate them. | |
| (Qi, 2016) [63] | This study tried to explain preschool teachers' public beliefs about ASD. | A total of 215 Undergraduate university students in Macau. | Completed self-report measures assessing two beliefs concerning autism spectrum disorder etiology: (1) A belief in parental factors and (2) a belief in genetic factors. | The result confirmed that belief in ASD etiology statement is caused by negligent and emotional parenting, while one-third of participants believed in genetic etiology. However, participants expressed mild to moderate agreement with statements describing paternity as etiology in ASD. | |
| (Riany, 2016) [64] | The aim of this study was to examine how Indonesian mothers understand autism and the appropriate ways to parent such a child. | Nine Indonesian mothers | Using semi-structured interviews with nine Indonesian mothers. | The interviews revealed five related themes about autism, including traditional cultural beliefs about appropriate behavior during pregnancy, karma, and God's plan, which is not usually reported in the literature from Western countries. | |

**Table 2.** *Cont.*

| Authors\years | Objective | Sample | Instrument | Result | Conclusion |
|---|---|---|---|---|---|
| (Hebert, 2010) [65]. | This article is a review paper focused on parents' beliefs about the cause and course of ASD. | The data were searched from 1995 to 2009; the keywords were autism, autistic disorder, belief, culture, parents, attitudes, and perceptions. | Review paper. | It was found in the review that parents hold a wide variety of beliefs about the cause of their child's autism, including genetic factors, events surrounding the child's birth, and environmental influences in the early childhood period. Some parents continue to attribute their child's autism to immunizations, although more recent studies suggest the frequency may be decreasing. Some parents are pessimistic about their child's future while others are hopeful that new strategies will be developed. | Some trust that society will become more accepting of their child's idiosyncrasies. Parents' beliefs about the cause of their child's autism have been found to have an impact on decisions regarding future health care, family planning, and maternal mental health. The link between parental beliefs and their choices for interventions has not yet been empirically explored. |
| (Khanam R., 2018) [66] | The study aimed to increase awareness of society and participants of the family about autism. | Various respondents including parents, family members, neighbors, relatives, and therapists from Dhaka, Bogra, and Jessor district. | Open-ended questionnaire survey on various respondents. | The response was explanatory and analysis was done on the summary. It was observed that social negligence and lack of understanding have a greater impact on the development of autistic children, as well as increasing the suffering and insecurity of parents. Ideas have been provided based on the results of the analysis. | |
| (Bazzano, 2012) [67] | The study aimed to assess how parents change and discontinue their child's vaccine schedule after their child is diagnosed with ASD, and assessment of how beliefs about the etiology of autism affect parents' decision to do so. | A total of 197 eligible parents of children under 18 years of age. | Survey. | The result of this study found that parents changed vaccination practices and this change was associated with a belief that vaccines contributed to ASD. | The study suggests that educational tools should be designed to assist physicians when speaking to parents of children with ASD about vaccination. |

Theoretically, the health belief model (HBM) can explain preschool teachers' beliefs, as this theory describes health-related behaviors and medical decision-making. The HBM was initially developed in the 1950s to explain why people did not participate in preventive disease programs [68,69]. Preschool teachers' beliefs can be a barrier preventing them from performing health behaviors for the children. Besides, the model could also explain how preschool teachers' beliefs affect their actions to protect children in the class and help them take the appropriate action [70].

The judgments of preschool teachers regarding the perceived barriers and the perceived benefits of an action define the course of the action taken; these two components together form the dimension of outcome expectations [70]. Preschool teachers' "perceptions of the costs involved in seeking a diagnosis" include time, not having evidence, social stigma, how to voice their concern to parents, not knowing who to contact, refusal of the parents, etc.

Moreover, some researchers have described the difficult behaviors as challenging, and these behaviors could lead to a delay in referral decisions over a more extended period. In fact, several teachers said that they were unwilling to allocate a stigmatizing label to the children and worried about the parents' reactions to their valuation of their child's problematic behavior. All of these pertain to the component addressed as perceived barriers [2,9]. As several studies have confirmed, ASD beliefs affect teachers' decision-making, finding that special education teachers agreed with common features and misconceptions of autism more than authentic reports of autism specialists [71]. The study also assessed teachers' and parents' belief and knowledge related to various aspects of ASD, finding that both groups had misbeliefs related to cognitive, developmental, and emotional aspects of ASD [71]. Both the teachers and the parents believed that children with ASD were mentally delayed but more often agreed that the children had special talents and were more intelligent than test detections [71]. Moreover, these misbeliefs could result in an overly high tendency for schools and homes to interpret the disorder as "stubbornness" instead of deficits in understanding or ability [71]. This misbelief is attributed to teachers' overestimation of children during diagnosis [72], becoming barriers that prevent teachers' from taking appropriate action.

These barriers of beliefs are defined as detrimental to self-efficacy in taking action, seeking diagnosis, and in making referral decisions [8] due to insufficient training, conflicts with specialists on interference, and a lack of referral plans [73].

Therefore, teachers must have accurate knowledge and beliefs about autism to meet the complex behavioral needs of children with autism. This situation is especially important, as some of the exceptional skills of students with autism may cause teachers to misinterpret students' social and learning skills, and consequently, provide insufficient support [74]. This issue could be addressed by providing the teachers with appropriate education and training.

### *1.3. ASD Identification Skills among Pre-School Teachers*

Skills are known as the "ability to do something well; in other words, it is the ability to use one's knowledge effectively and readily in behavioral execution or performance" [75]. In this study, skills are referred to as the preschool teacher's skills to identify children with ASD by implementing their knowledge regarding the risk and symptoms of ASD.

Most people perform early screening and identification of ASD utilizing the knowledge and skills of early childhood specialists or through persons with daily constant contact with the child such as preschool teachers [76]. However, several factors act as barriers to preschool teachers' identification or decision-making and then referral of the child for early intervention [9]. One of these barriers include skill [14,16,19,20,77,78]. There is a specific skill to elicit and recognize early markers of ASD [36]. Thus, preschool teachers need to improve on skills in observing growing children, recording their behavior, and lastly writing a report.

Furthermore, these skills need to be determined to give teachers the necessary skills to influence parents, collaborate with them, and negotiate an appropriate referral system [9]. Some studies have confirmed that preschool teachers' lack of knowledge and skills have impacted the management and

determination of behavioral problems among abnormal children with ASD [19,20]. Therefore, several preschool teachers have expressed the need for more training to handle challenging behaviors such as ASD [20,79].

This study aims to provide preschool teachers with important identification skills to help them identify children suspected with ASD. These identification skills are observation skills, recording skills, and report-writing skills (Table 3).

Observation is the process of looking at a child at work or play without coming off as nosy. It is considered an essential tool for acquiring information, gaining results, and generating ideas. Recording is defined as one of the observation skills involving writing down an observed activity or behavior. Although several teachers are used to recording, a regular method will guarantee that the children are properly observed while participating in many different activities at a time. With these steps, preschool teachers will be able to describe the child's behavior and write down important information.

**Table 3.** Summary studies of identification skills about ASD.

| Authors\years | Objective | Sample | Instrument | Result | Conclusion |
|---|---|---|---|---|---|
| (Yasin M., 2020) [80] | This study aim is to identify teacher strategies and ability in identifying students with special needs. | Primary teachers. | This mixed method study involves 16 respondents in a qualitative study and 219 respondents in a quantitative study. | The study found that 50.2% of respondents achieve mastery level while 49.8% achieved less than mastery level. The study also found the ability to identify children with special education needs (SEN) based on their external behavior. Therefore, the qualitative study found that most of the teachers can identify children with disabilities through children's behaviors and characteristics, while some of the respondents identify children based on academic performance, including children's abilities to read and write. | |
| (Rosenbaum, 2019) [81] | The study aimed to understand pre-referral perception and decision factors involved. | Among 346 teachers. | [80] | The study found decision factors linked with play, social interactions, engagement, and verbal behaviors, but none were cited by a clear majority. | |
| (Splett J., 2019) [82] | The study examined the ability of teachers to accurately identify mental health concerns among elementary children. | A total of 153 teachers. | Vignette scenarios. | Findings indicated that teachers could accurately identify children with severe externalizing and internalizing problems. However, they were less accurate and less likely to think children with moderate or subclinical symptoms needed services. | |
| (Gabrielsen, 2019) [81] | The study aimed to better understand pre-referral perceptions and decision factors involved. | A total of 364 teachers and clinicians. | Multiple video clips from early signs of autism; the teachers and clinicians were asked to evaluate the child and to make decisions about ASD referral. | The result found that decision factors linked most often with play, social interaction, and verbal behaviors. | The study result confirmed the need for training in early childhood professionals; targeted training may encourage earlier referrals when autism is suspected in young children. |

**Table 3.** *Cont.*

| Authors\years | Objective | Sample | Instrument | Result | Conclusion |
|---|---|---|---|---|---|
| (Smith M., 2017) [83] | This study investigated whether teachers can recognize children's anxiety and somatic symptoms, and how they identify children they perceive to be anxious or somatizing. | A sample of 1346 seven- to 11-year-old children, their 51 class teachers, and 144 parents took part in the study. | Data on children's anxiety and somatic symptoms were collected using standardized scales and simple 1–5 teacher rating scales. Teachers were also asked to identify children they perceived to have "debilitating" levels of anxiety and (separately) somatic symptoms and to provide brief qualitative descriptions to explain their choices. | Small but significant positive associations were found between teachers' and children's reports of anxiety and somatic symptoms. Identified children reported similar levels of anxiety than children not identified, but significantly greater levels of somatic symptoms, although the size of this difference was modest. Teachers commonly described crying and avoidance as signs of anxiety. | Findings suggest that teachers show limited sensitivity to the variation in pupils' levels of anxiety and somatic symptoms, and may struggle to identify children who may benefit from interventions or extra support in these domains. |
| (Deyessa A., 2017) [31] | The study examines teachers' ability to identify children's "debilitating" levels of anxiety and (separately) somatic symptoms and to provide brief qualitative descriptions to explain their choices. | Ethiopian teachers. | Data on children's anxiety and somatic symptoms were collected using standardized scales and simple 1–5 teacher rating scales. | The result indicates that a teacher's training was significantly associated with more accurate identification of a child. | |
| (Drusch, 2015) [8] | The study attempted to understand whether preschool teachers are familiar with signs of ASD in young children and their ability to discuss concerns with a child's parents, and preschool teachers' knowledge about diagnosis and intervention services in ASD. | Eighty-four preschool teachers. | | The study result found preschool teachers have a moderate level of knowledge regarding ASD symptoms based on teachers' experiences. Also, preschool teachers held positive perceptions about mainstreaming and those who have had training specific to inclusion.Teachers with greater experience reported comfort to express their concerns with a child's parents. | Confirmed that professional or personal experience is not enough to increase a teacher's knowledge and skills. In a similar vein, teachers cannot gain knowledge and skills in identifying and recognizing ASD just by working with children. |

In the social cognitive theory, Pajares [51] confirmed that self-efficacy cannot lead to requisite behavior when skills and knowledge are lacking. Instead, "competent functioning requires harmony between self-beliefs on the one hand and the possession of skills and knowledge on the other" (p. 3) [53]. To foster competent functioning development among preschool teachers, the teachers must have correct understanding, knowledge, and skills regarding ASD, especially given their critical role in supporting effective teaching and referral [84,85]. To assess self-efficacy, a person's specific skill sets or particular skills related to particular challenges or topics must be evaluated. Hence, when preschool teachers are exposed to education or training programmes on knowledge and skills, the most significant outcome will be increased self-efficacy [36]. Drusch [8] confirmed that professional or personal experience is not enough to increase a teacher's knowledge and skills. In a similar vein, teachers cannot gain knowledge and skills in identifying and recognizing ASD just by working with children. Instead, the teachers need to be exposed to more training and equip themselves with the skills to recognize ASD symptoms among children. In doing so, they will increase their ability to refer these children to the appropriate services.

### *1.4. ASD Self-Efficacy among Preschool Teachers*

Self-efficacy is one of several teacher-related characteristics that is consistent with effective teaching practices, classroom learning, and communication with children [11]. According to Bandura's [86] theory of social cognition, self-efficacy refers to an individual's belief (i.e., one's confidence in one's competency to do a particular task) and has the most powerful effect on the behavior and motivation of the individual. However, this power is linked to other variables such as knowledge, skills, beliefs, attitude, and the individual's intention [44]. Based on Bandura's [60] definition, self-efficacy refers to preschool teachers' beliefs about their ability to successfully perform a particular behavior [36,87,88]. Moreover, a high sense of self-efficacy is positively related to promising results. For example, it is associated with encouraging in-class behavior, classroom practice, use of praise more often than criticism, increased perseverance with "low achievers," spending more time monitoring student performance, and spending more time on class preparation and paperwork, increased willingness to collaborate with other professionals regarding student concerns, increased significant effort, and increased success [87–91].

Several researchers claim that high-level knowledge among teachers could correlate to increased self-efficacy. For instance, Sasson [36] found a significant correlation between knowledge and self-efficacy among allied health professionals. The study noted that increased knowledge in ASD could increase the clinical confidence of health professionals. Bandura [60] confirmed these findings, stating that both knowledge and performance are thought to affect self-efficacy [44]. Therefore, studies have turned to teacher training to explain the gap in study and practice. Moreover, based on Bandura's [60] theory, teachers who believe in their ability to address behavioral problems such as ASD would work towards making a difference for those children (p. 560). Hence, self-efficacy is one of the most crucial factors affecting whether or not the teachers will apply classroom-based training programs or educational modules in early childhood development and whether or not they would be able to identify problematic behavior such as ASD [92].

As it is known, ASD is a childhood development disorder (CDC, 2013) [93]. Hawley and Williford [94] identified children with ASD as requiring teachers with high self-efficacy so that the likelihood that these children are transferred to intervention services is increased [94]. Furthermore, a high sense of self-efficacy among preschool teachers would cause them to recognize children with ASD as a complex problem. However, teachers who lack the knowledge to recognize the behavioral problems of ASD [8] will not have high-level self-efficacy and would not be confident in recognizing children with ASD [36]. For more details about the literature review in self-efficacy in ASD, see Table 4.

**Table 4.** Summary studies of self-efficacy about ASD.

| Authors\years | Objective | Sample | Instrument | Result | Conclusion |
| --- | --- | --- | --- | --- | --- |
| (Sasson, 2018) [35] | To examine the effect of an early screening training on pediatric Physical Therapists PTs': (1) Knowledge of autism spectrum disorder (ASD), (2) clinical self-efficacy, and (3) identification of markers. | Twenty-six pediatric PTs participated in a two-day 'Early ASD Screening' workshop. | Questionnaire in both ASD knowledge and self-Efficacy, and video case study. | The result confirmed that there is an increase in PTs' knowledge and self-efficacy before and after the ASD workshop, and the PTs ability to identify the early signs of ASD is greater than before the workshop. | Conclude that the workshop is useful to increase the level of knowledge and self-efficacy among PTs. |
| (Drusch, 2015) [8] | The study attempted to understand whether preschool teachers are familiar with signs of ASD in young children and their ability to discuss concerns with a child's parents, and preschool teachers' knowledge about diagnosis and intervention services in ASD. | Eighty-four preschool teachers. | | The study result found pre-school teachers have a moderate level of knowledge regarding ASD symptoms based on teachers' experiences. Also, preschool teachers held positive perceptions about mainstreaming and those who have had training specific to inclusion. Teachers with greater experience-reported comfort to express their concerns with a child's parents. | Confirmed that professional or personal experience is not enough to increase a teacher's knowledge and skills. In a similar vein, teachers cannot gain knowledge and skills in identifying and recognizing ASD just by working with children. |
| (Arslan, 2017) [95] | This study investigates the effect of preschool teachers' collective self-efficacy. | A study group consists of 172 preschool teachers who are working in public preschools affiliated with the Ministry of National Education in different cities of Turkey. | In this study, the teacher self- efficiency scale is employed to assess professional efficiency; it was found that there was a positive relationship between teachers' self-efficacy and collective self-efficacy. | The study found that teachers' self-efficacy can significantly explain collective self-efficacy. | Proficiency is the ability to have the professional knowledge, skills, and attitudes required to carry out tasks specific to a profession. In-service training activities for teachers will enable them to improve themselves in various subjects and providing training enabling them to benefit from their professional knowledge will contribute because, according to this research, the increase in the sense of occupational competence leads to the increase in collective self-efficacy levels. |

**Table 4.** *Cont.*

| Authors\years | Objective | Sample | Instrument | Result | Conclusion |
|---|---|---|---|---|---|
| (Gascoigne, M., 2019) [96] | This article aims to evaluate the efficacy of a brief in-service training workshop at increasing primary school teachers' ADHD knowledge and sense of self-efficacy. | Teachers from 10 schools participated in the study (n = 274) and were allocated into either an intervention or waitlist control group. Teachers'ADHD knowledge and self-efficacy were assessed following the provision of a brief training workshop on ADHD. Knowledge and self-efficacy retention was also assessed at a one-month follow-up. | | Results: Within the intervention group, ADHD knowledge and self-efficacy increased following the intervention. ADHD knowledge increased more than twofold, from very low to high levels, although increases in self-efficacy were more modest. Both knowledge and self-efficacy decreased at the one-month follow-up but, nevertheless, remained higher than baseline levels ($p < 0.001$). | Results demonstrate that a brief training workshop can increase primary school teachers' ADHD knowledge. |

Numerous evidence has confirmed the association between one's self-efficacy and confidence in one's ability to carry out a task [8,9,36]. However, some studies found teachers to be generally confident in their ability to deal with children with ASD, while others found that teachers had a low level of confidence regarding special-needs children suggesting they need more training in special education [97]. In line with Bandura's [60] theory, a teacher with more knowledge and training specific to catering to children with ASD would have higher self-efficacy to deal with the affected children and could identify them early on [9].

In this study, preschool teachers are defined as having two types of self-efficacy: (1) The ability to discuss with parents and counselors, and (2) the confidence to help the diagnostic team.

### *1.5. Theoretical Rationale*

The conceptual framework developed in this study was based on two theories: Bandura's [60] social cognitive theory (SCT) and Rosenstock et al.'s [70] health belief model (HBM).

#### 1.5.1. Social Cognitive Theory (SCT)

This theory was initially called social learning theory (SLT) when introduced in the 1960s by Albert Bandura. Later, the theory was renamed social cognitive theory in 1986; positing that learning occurs in a social context with a dynamic and reciprocal interaction between the person, environment, and behavior.

One unique feature of SCT is its emphasis on social influence and external and internal social reinforcements. SCT considers the unique way in which individuals acquire and maintain behavior, while also considering the social environment in which individuals perform the behavior. The theory takes into account a person's past experiences, which factor into whether behavioral action will occur. These past experiences influence reinforcements, expectations, and expectancies, all of which shape whether or not a person will engage in specific behavior and the reasons why a person engages in that behavior [98].

Many theories of behavior used in health promotion do not consider the maintenance of the behavior, but rather focus on initiating the behavior. This is unfortunate, as the maintenance of the behavior, and not just the initiation of the behavior, is the true goal of public health. The goal of SCT is to explain how people regulate their behavior through control and reinforcement to achieve goal-directed behavior that can be maintained over time. The theory provides a framework for understanding how people actively shape and are shaped by their environment. In particular, the theory details the processes of observational learning and modeling, and the influence of self-efficacy on the production of behavior [99].

Initially, Bandura [60] developed five constructs. Later on, the self-efficacy construct was added when the theory evolved into SCT. These constructs and how they relate to this study are explained in detail below:

1. Reciprocal determinism—this is the central concept of SCT that refers to the dynamic and reciprocal interaction of a person (in this case, preschool teachers with a set of learned experiences, level of education), environment (external social context), and behavior (responses to stimuli to identify children with ASD).
2. Behavioral capability—this refers to a preschool teacher's actual ability to perform a particular behavior (to identify children with ASD) through essential knowledge and skills. To successfully perform the behavior, preschool teachers must know what to do and how to do it. Preschool teachers learn from the consequences of their behavior, which also affects the environment (class) in which they work.
3. Observational learning—this asserts that preschool teachers can witness and observe behavior conducted by others, and then reproduce those actions. This is often exhibited through the

"modeling" of behaviors. If preschool teachers see the successful demonstration of a certain behavior, they can also complete the behavior successfully.

4. Reinforcements—this refers to the internal or external responses to a preschool teacher's behavior that affect the likelihood of continuing or discontinuing the behavior. Reinforcements can be self-initiated or originate from the environment, and reinforcements can be positive or negative. This is the construct of SCT that most closely ties into the reciprocal relationship between behavior and environment.
5. Expectations—this refers to the anticipated consequences of a preschool teacher's behavior. Outcome expectations can either benefit or not. Preschool teachers anticipate the consequences of their actions before engaging in certain behavior, and these anticipated consequences could influence the successful completion of the behavior. Expectations derive largely from previous experience. While expectancies are also derived from previous experience, expectancies focus on the value that is placed on the outcome and is subjective to the individual.
6. Self-efficacy—this refers to the level of preschool teachers' confidence in their ability to successfully perform a certain behavior. Self-efficacy is unique to SCT although other theories have added this construct at later dates, such as the theory of planned behavior. Self-efficacy is influenced by preschool teachers' specific capabilities and other individual factors, as well as environmental factors (barriers and facilitators).

#### 1.5.2. Health Belief Model (HBM)

HBM is a theoretical study that describes health behavior and medical decision-making skills. The original HBM was established in the 1950s. The model focuses on the behavior of individuals who have declined to participate in preventive disease programs [68,70]. The model has been implemented in several works aiming to study patient behaviors like dieting in obese children, factors of protection from skin cancer, and parenting skills programs to enroll associated parents with parental motivation [100–102]. However, HBM usually uses an individual's health behavior. In this study, HBM was used to describe preschool teachers' beliefs and how their actions predict the voicing of their concerns about children's health [103]. HBM contains six components under four factors (threat exception-outcome, exception-self efficacy, exception-cues to action) [70]. Perceived barriers are one of the concepts under outcome exception. It refers to the barriers that prevent the preschool teachers from taking a particular action or voicing out his or her concern to the suspected child's parents. These barriers include social stigma, not knowing who to contact to refer to, waitlists, etc. The perceived barriers held by preschool teachers can impede their identification of ASD and subsequently their referral decision [2,9]. An example of the preschool teachers' barrier is that they believe ASD among children is normal and that children's behavior will change as they mature [19].

Moreover, family culture and language differences are also considered as barriers that influence teachers' ability to make a referral for a child with ASD [14]. Another source of reluctance in making referrals or discussions with parents is teachers' negative perceptions and concerns about the parents, in turn, influencing the teachers' ability as well. Several teachers are uncomfortable expressing their concerns that a child has ASD to a child's parents because of parents' reactions, as some parents attribute a stigmatizing label to ASD. Another study confirmed that teachers found it easier to tell parents that their children had verbal and language problems rather than a mental disorder, as the former is less stigmatizing and would have less potentially negative repercussions [104]. Ultimately, teachers' concerns have a direct effect on their ability to take action on behalf of children with ASD. Concerns about labeling and communicating with parents may be closely related [14].

In the end, preschool teachers' self-efficacy may play a role in their identifying and making decisions to proceed, as they pursue answers about their concerns for the children's development to ultimately obtain an early diagnosis of ASD. Some studies have identified factors relating to teachers' knowledge, observation skills, and their belief about young children with ASD that could influence their decisions to make referrals [14,15].

## 2. Methodology

The present study reviewed the literature and theories in-depth to look for evidence of factors that could improve preschool teachers' ability to identify children with ASD. The study focused on some variables to enhance teachers' knowledge and to change their beliefs regarding ASD overall and the early signs of ASD specifically, and to equip them with identification skills in ASD, besides enhancing their self-efficacy in identifying children with ASD.

## 3. Result

According to an in-depth literature review, preparation of preschool teachers becomes an essential step to support early diagnosis through early identification in preschool. Preparation of preschool teachers should be done by building an educational module. Therefore, according to the results of the present study, it is evident that several elements can be used to prepare preschool teachers to identify children with ASD. These elements include knowledge and beliefs, identification skills, and self-efficacy, which this study conceptualized as essential elements for the proposed framework, with suggestions for an educational module besides experimental testing as a valuable contribution to the literature.

*Conceptual Framework Development*

The conceptual framework aims to prepare preschool teachers to identify children with ASD. The conceptual framework contains several variables, namely knowledge in ASD; identification skills in ASD; belief in ASD; and self-efficacy in identifying children with ASD. Some variables have several sub-variables, as shown in Figure 2.

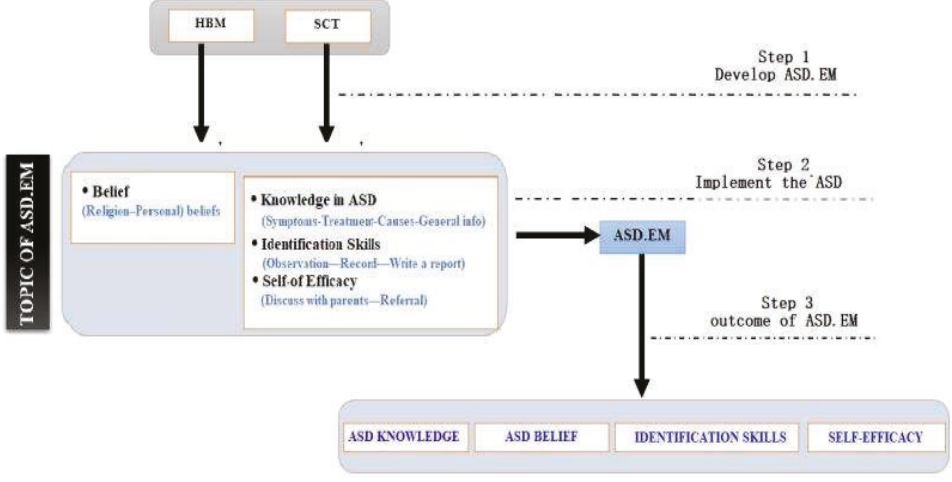


**Figure 2.** A conceptual framework for identifying children with autism spectrum disorder (ASD).

One of the barriers to ASD identification is preschool teachers' lack of knowledge in the area of ASD [8,21,105]. Knowledge of ASD here refers to preschool teachers' knowledge and information about ASD, symptoms of ASD, its causes and treatment. Preschool teachers could be exposed to these variables via an educational module. Based on SCT, increased knowledge among preschool teachers regarding ASD signs will increase their self-efficacy in identifying children with ASD [36,97,104].

Secondly, identification skills refer to a particular part of this conceptual framework relating to the identification skills that preschool teachers needed to improve upon. These skills are observation, recording, and reporting (see Figure 3).

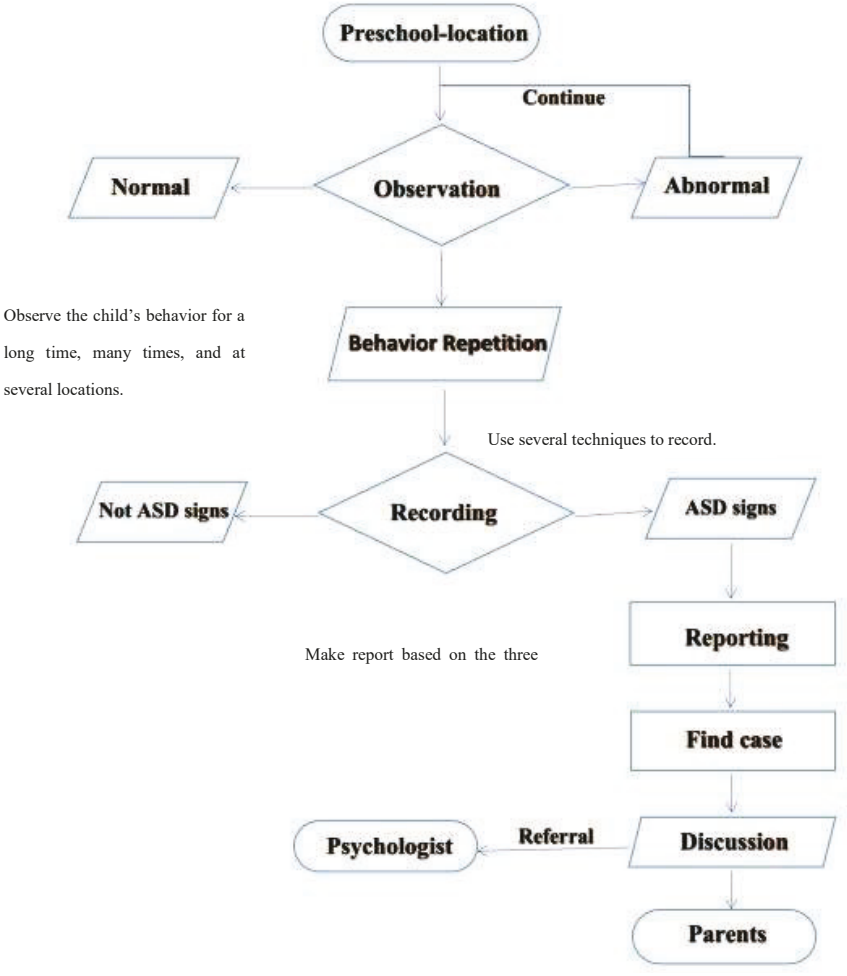


**Figure 3.** Identification skills process.

As shown in Figure 3, in the preschool location, teachers can exercise their identification skills by following the steps below:

The preschool teacher observes the children under her care under several situations in class, outdoors, during teaching activities, and/or playing activities. In case a child exhibits abnormal behaviour, the preschool teachers move to the next step of recording the behaviour in different locations using different technical skills. The preschool teachers should be looking for ASD warning signs in communication and social interaction, and patterns of behaviour, and interests in their activities. If the preschool teacher observes the behaviour repeated many times, she should record her observation and move to the third step, which is to write a report [106]. After the preschool teacher determines the suspected child, she should share her concerns on the atypical developments with a psychologist or the child's parents or both and show them the reported behaviour and ask them to refer the child to a specialist if necessary [107].

Thirdly, the belief variable refers to teachers' feelings and concerns regarding children's behaviour that influence their identification and voicing out concerns to children's parents, their referral decisions, and the timing of their decision. This belief is divided into three categories (religious, societal,

and personal). These variables are considered the third barrier preventing preschool teachers from identifying ASD. Also, the hypothesis underlying these variables states that if inaccurate beliefs among preschool teachers are reduced, their ability to identify ASD will increase and they will be more confident to voice their concerns with children's parents.

Finally, self-efficacy in identifying children with ASD is a variable that refers to preschool teachers' ability to identify ASD symptoms, discuss with parents, and the confidence to make referral decisions [8]. The main goal of this framework is to enhance preschool teachers' ability to identify children with ASD in preschool. Therefore, a hypothesis is proposed that if preschool teachers have a high level of preparation in identifying children with ASD, their ability to determine the red flags indicating ASD among children will increase. In turn, teachers will improve their ability to voice their concerns to the child's parents, and ultimately, they can make the decision to refer the child for formal diagnosis and then for early intervention.

## 4. Discussion

This study is a concept paper, that presents a discussion on preschool teachers' ability to identify children with ASD via a review of past studies and existing theories to develop a conceptual framework. This conceptual framework helps preschool teachers prepare to identify children with ASD based on different variables such as knowledge in ASD, belief in ASD, identification skills, and preschool teachers' self-efficacy in identifying children with ASD.

This study aimed to determine the association between knowledge and identification of children with ASD. Preschool teachers' knowledge in ASD is considered the most important factor that helps them identify early signs of ASD. Preschool teachers cannot take action without having basic information about the disorder [50]. Besides, preschool teachers must be educated in the early signs of ASD to be able to deal with this kind of behavioral disorder [16]. Also, the barriers preventing teachers from detecting ASD at an early stage should be removed.

On the other hand, this study discussed other barriers preventing preschool teachers from identifying children with ASD, one of which is preschool teachers' beliefs. These beliefs are identified as barriers preventing them from identifying children with ASD. Preschool teachers cannot take action or refer the parents of the child suspected with ASD to specialists because some parents still perceive ASD as a stigma. Moreover, some preschool teachers still have misbeliefs and often attribute this disorder to "stubbornness" instead of deficits in mental ability [71]. As per the health belief model (HBM) incorporated in the conceptual framework of this study, these barriers among preschool teachers could be reduced, as several studies have confirmed [2].

Also, preschool teachers' competent functioning development must be aligned with correct understanding, knowledge, and skills, especially given the critical referral role that they have [84,85]. The specific skill sets or particular skills related to particular challenges or topics such as teachers' identification of children with ASD should be evaluated. This study proposed examining teachers' self-efficacy to explain their ability to do so [36].

Preschool teachers must have the ability to refer children suspected with ASD to specialists and discuss the issue with children's parents. Therefore, they must have knowledge of early signs of ASD, correct belief, and ASD identification skills. Preschool teachers must have the self-efficacy to identify children with ASD. However, more than a few studies have mentioned insufficient resources such as training programmes or educational modules to educate preschool teachers in identifying children with ASD [16]. So, this study suggested using an educational module to address these challenges. Also, the study framework will help improve preschool teachers' ability to identify children with ASD, as confirmed by several studies [36,59].

## 5. Conclusions

This study offered a specific emphasis on the early identification of children with ASD, which will further improve early diagnosis and early intervention for the children. Furthermore, the lack of

knowledge and incorrect beliefs among preschool teachers and parents should be closely examined to increase the percentage of children that obtain correct diagnoses within the appropriate time, as this would help to significantly impact the children's actual behavior.

It is important to highlight the limitations of this study. Firstly, the approach of this study was to build a conceptual framework that may not be backed by experimental work. Secondly, other factors may affect preschool teachers' ability to identify ASD but were not discussed in this study, such as experience working with ASD, intention, and attitude. Thus, this study calls for a more qualitative and quantitative approach to assess the factors that can increase preschool teachers' self-efficacy in making decisions and referring children with ASD for diagnosis. Moreover, the benefit of the proposed module in the long term was not examined in this study. Moreover, this study did not focus on parents with ASD children. Therefore, future works should focus on preparing parents in identifying whether or not their children have ASD to support early detection and early intervention at an early stage.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Review*

# Exclusion Criteria Used in Early Behavioral Intervention Studies for Young Children with Autism Spectrum Disorder

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Received: 16 January 2020; Accepted: 11 February 2020; Published: 13 February 2020

**Abstract:** This literature review evaluated early behavioral intervention studies of Autism Spectrum disorder (ASD) based on their participant exclusion criteria. The studies included were found through searching PsycINFO and PubMed databases, and discussed behavioral interventions for children up to 5 years of age with ASD and utilized a group research design. Studies reviewed were categorized into three groups: Restrictive exclusion criteria, loosely defined exclusion criteria, and exclusion criteria not defined. Results indicated that studies that used restrictive exclusion criteria demonstrated greater differences in terms of outcomes between experimental and control groups in comparison to studies that used loosely defined exclusion criteria and/or did not define any exclusion criteria. We discussed implications for the generalizability of the studies' outcomes in relationship to exclusion criteria.

**Keywords:** autism spectrum disorder; autism; literature review; comorbidity; early intervention; early intensive behavioral intervention; behavioral intervention

## 1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that involves impairments in social communication, as well as the presence of stereotyped patterns of behaviors and interests [1]. ASD is considered a leading cause of disability in children under 5 years of age [2]. Given that ASD affects approximately 1 in 59 children in the United States [3], it is considered a serious public health concern [4]. This higher prevalence may be partially due to better detection and assessment procedures and an expanded definition of ASD [3,5,6].

While in the past, children with ASD were typically diagnosed around the age of 4 years shortly before entering school, they are now being diagnosed as early as the age of 2 years [7,8] and identified as at-risk for ASD between 12 to 24 mon of age [9]. With the increase in the number of young children being diagnosed, developing early age-appropriate interventions that can support parents and children is an international clinical and research priority [10,11].

Currently, research evidence indicates that high-intensity, long-term behavioral interventions are the most efficacious in supporting development and diminishing ASD symptoms and associated disabilities [12–17]. In a seminal study on behavioral intervention for children with ASD, Lovaas [14] demonstrated that children aged 40 to 46 mon who participated in intensive, long-term applied behavior analysis therapy achieved remarkable improvement in their skills. Specifically, nearly half of the children enrolled in intensive applied behavior analysis (for a minimum of 40 h per week), for at least 2 years showed significant gains in their adaptive and intellectual functioning, with some children becoming nearly indistinguishable from their typically developing peers. At long-term follow-up,

the children who made significant gains maintained those gains, with placement in mainstream classrooms. This study led to widespread interest in behavioral interventions as promising treatments for children with ASD, spurring the development of educational treatment programs [18].

Despite the promising results found in the Lovaas [14] study, there was variability in the functioning of the study participants, with 40% of the participants continuing to meet criteria for developmental delays and needing educational supports. Replication of the Lovaas [14] study provided partial support for the treatment gains achieved, but with some disappointing results as the gains made during the replication were not as robust as the original study [18,19]. The variability in the results of the Lovaas [14] study have been related to variability in the severity of the study participants' ASD symptomatology, with participants with Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS), a former diagnosis that included fewer symptoms than ASD, showing better outcomes than study participants that met full criteria for ASD [18].

Despite the positive impact of early intervention for preschoolers with ASD (age 12–72 mon), response to the intervention program is variable [18,20]. Outcomes for preschoolers who received early intervention range from loss of diagnosis to lack of improvement in the core ASD symptoms, from dramatic gains in language, cognitive, and adaptive skills to minimal treatment gains [21]. There are at least two possible reasons for the variability in the outcome of early-intervention studies. First, most studies do not describe the sample characteristics in detail. Even less is mentioned about the social and demographic factors that might influence the outcome [22]. Second, is the clinical heterogeneity of autism [23]. Despite the current custom of conceptualizing autism as a spectrum disorder following the publication of fifth edition of The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [1], it may be the case that subtypes exist within the autistic spectrum [24].

In addition to the possible subtypes of autism, several medical and behavioral conditions are known to co-exist with it. It is estimated that approximately 75% of individuals with ASD present with associated medical conditions, genetic syndromes, or mental health disorders [3,25]. On the other hand, in some studies, due to the attempt to recruit homogeneous samples of individuals with "pure" ASD, children with associated conditions such as epilepsy, severe intellectual disabilities, or genetic abnormalities, are not included [12]. Many studies also used small clinical samples or lacked details about the ASD characteristics that lead to diagnosis [22].

Thus, by excluding persons with ASD who have associated medical and behavioral disorders, who constitute the majority of the general ASD population [26], these stringent exclusion criteria significantly reduce the generalizability of results and reduce their utility in the real world. Without knowing the characteristics of the children who benefit from the intervention, it is difficult to make treatment recommendations in clinical practice. This review aimed to examine the exclusion criteria used in the early-intervention studies of ASD, in order to ascertain how these criteria are related to the efficacy of behavioral interventions for young children with ASD.

## 2. Materials and Methods

Our review included 26 papers written between 2002 and 2018 that highlighted studies with three varying levels of exclusion criteria used in early behavioral interventions for children with ASD. PubMed and PsycINFO were the databases used to identify articles included in this review. Search terms used included various combinations of the following terms: "Early intervention", "Autism", "Autism Spectrum Disorder", "children with autism", "children with ASD", "clinical trial", and "group design". A filter limiting the results to publication years of 2002 to 2018 was applied. Other studies were found from the reference list of the articles that met these inclusion criteria. The search was conducted through December 2018.

The titles and abstracts of these studies were reviewed by the first, second, and fourth authors for appropriateness to include in the literature review, particularly for the inclusion of a behavioral intervention and the age of study participants. Inclusion criteria for this review were studies that (1) used participants between the ages of 2 and 5 years with Autism Spectrum Disorder, (2) investigated

a behavioral intervention, (3) used a group design, and (4) were published within the last 15 years. Group studies were the focus of this review so that comparisons could be drawn among studies. Early behavioral intervention was another focus of this review, which is why studies that only used young participants and behavioral intervention were included. Given the increasing prevalence of ASD and corresponding treatments, the focus was also on studies recently published. Studies using both DSM-IV-TR [27] and DSM-5 [1] criteria were included, as there were few studies using DSM-5 criteria. Studies that employed single-case design and nonbehavioral interventions, such as dietary and pharmacological interventions, were excluded. No language filters were applied, but only one study was excluded for being in a language other than English.

## 3. Results

There were 26 studies found based on the search methods and inclusion criteria specified above, published between the years of 2002 and 2018. For this review, the term "restrictive exclusion criteria" categorizes studies that excluded children with comorbidities and/or associated family mental health conditions. The term "loosely defined exclusion criteria" defined studies that included children with comorbidities but excluded certain individuals on the basis of other factors, such as distance of the family from the treatment center, non-English-speaking participants, or severe sensory or motor deficits. The term "exclusion criteria not defined" highlighted studies that did not significantly excluded any children. A summary of all studies can be found in Table A1.

### 3.1. Restrictive Exclusion Criteria

Of the studies, 57% ($n$ = 15/26) used comparably restrictive exclusion criteria to select their participants. Studies with this type of restrictive criteria mainly excluded participants with medical conditions other than ASD, such as genetic syndromes, epilepsy, and intellectual impairments.

Perera, Jeewandara, Seneviratne, and Guruge [28] investigated an early-intervention program for children aged 18 to 40 mon in Sri Lanka. Study participants were children who had just received an initial diagnosis of Autism, were 18 to 40 mon in age, and had never received behavioral or developmental intervention previously. Participants were excluded if they had a diagnosis of PDD-NOS or Asperger's Disorder, had severe cognitive impairments, experienced co-occurring sensory or motor disorders, genetic disorders, or if they had participated in developmental intervention prior to joining the study. Experimental group participants received home-based therapy in which their mothers were taught to use developmental and behavioral interventions to use with their children. Participants in the comparison group had received a diagnosis of autism over the age of 40 mon and did not receive any autism-specific developmental intervention. This study did not use random assignment. Results indicated that the children in the experimental group showed more improvement on measures of autism severity and social interaction, despite some improvement in the children in the comparison group.

Brian, Smith, Zwaigenbaum, and Bryson [29] conducted a cross-site, randomized, controlled trial investigating the efficacy of a parent-mediated intervention, social ABCs, for toddlers aged 16 to 30 mon with suspected or confirmed ASD. Exact numbers of male and female participants were not given. Inclusion criteria included children who met criteria for ASD or displayed behaviors consistent with ASD, did not spend more than half their time in childcare, were products of full-term delivery, and had a birthweight above 2500 g. Exclusion criteria included the occurrence of any co-occurring genetic, neurological, or severe sensory or motor conditions. Results indicated that children in the treatment group showed more gains in functional vocal responsiveness to parent prompts and child vocal initiation as compared to the control group.

Rogers et al. [30] conducted a randomized controlled trial with 98 children (76 boys) aged 12 to 24 mon. The study strove to investigate the efficacy of the Early Start Denver Model (ESDM), which fosters parental involvement within a child-centered interactive context and may be compared to conventional community therapies. Inclusion criteria specified that the children met risk criteria for ASD in a clinical assessment, were ambulatory, had a development quotient of 35 or higher, and

primarily spoke English at home. The exclusion criteria included children who had parents that self-reported mental illness or substance abuse, children who had significant medical conditions such as cerebral palsy, a gestational age of less than 35 weeks, and/or genetic disorders related to developmental disabilities, or individuals who had current or prior enrollment in an intensive 1:1 autism intervention curriculum for more than 10 h per week. The main outcomes of this study were that individuals who had received parental training with the ESDM technique established more productive working alliances with their therapists as compared to the community group. However, the effects seen in intensive-treatment studies were not observed. They demonstrated that younger age and greater intervention positively affected the developmental rates for children with autism.

Carter et al. [31] conducted a study with 62 children (51 boys) aged 15 to 25 mon. The study aimed to investigate the efficacy of Hanen's More Than Words (HMTW), a parent-implemented intervention, as compared to a control group. The inclusion criteria required the children to meet the diagnostic criteria of ASD and to be recruited from ASD specialty clinics. Children with a genetic disorder, those who did not obtain a predetermined "at-risk" score on the Screening Tool for Children with Autism (STAT), or those who did meet the symptom criteria for an ASD diagnosis based on clinical evaluations were excluded. The main outcomes of this study were that the HMTW group showed differential effects on child communication. However, parents of children who possessed higher object interest may require additional support to implement proper strategies.

Dawson et al. [12] evaluated the efficacy of the ESDM with a sample size of 48 children aged 18 to 30 mon. Exact numbers of male and female participants were not given, but the ratio of males to females was 3.5 to 1. The inclusion criteria for this randomized controlled trial stipulated that the children meet criteria for ASD on the Toddler Autism Diagnostic Interview and Autism Diagnostic Observation Schedule (ADOS), receive a clinical diagnosis for ASD based on DSM-IV criteria, reside within half an hour of the testing location, and demonstrate a willingness to participate in a two-year or greater intervention program. Children who had a neurodevelopmental disorder of known etiology, significant sensory or motor impairments, major physical problems such as chronic or serious health conditions, seizures at the time of entry, use of psychoactive medication, a history of serious head injury or neurological disease, alcohol or drug exposure during the prenatal period, or developmental quotient below 35 were excluded. The main outcomes of this study were that the children who received ESDM training demonstrated significant improvements in IQ scores and adaptive behavior and were more likely to have a change in diagnosis to pervasive developmental disorder. Moreover, the comparison group manifested greater delays in adaptive behaviors and demonstrated minimal improvement in baseline scores.

Kasari, Gulsrud, Wong, Kwon, and Locke [32] aimed to identify if a joint attention intervention would result in greater engagement between caregivers and toddlers with autism. The randomized controlled trial investigated 38 children (29 boys), aged 21 to 36 mon. Inclusion criteria stated that children must have met criteria for autism following DSM-IV criteria by an independent clinician; children with additional syndromes were excluded. The main outcomes were that both caregivers and toddlers in the experimental group made significant improvements in areas of joint engagement, including responsiveness to joint attention and diversity of functional play acts, as compared to the control group.

Zachor and Itzchak [33] compared the efficacy of applied behavior analysis (ABA) and the integration of several intervention approaches for children with varying levels of autism severity. The quasi-experiment investigated a sample size of 78 (71 boys), aged 15 to 35 mon. Participating children had to meet a clinical diagnosis of autism based on DSM-IV criteria and the cut-off points on the ADI-R (Autism Diagnostic Interview-Revised); those with additional major medical diagnoses or incomplete post-intervention assessments were excluded. While there were no significant between-group differences in terms of improved cognitive abilities or adaptive skills, Zachor and Itzchak demonstrated that in the group with less severe baseline ASD symptoms, the children who had

received the eclectic intervention approach had better outcomes in communication and socialization adaptive skills.

Itzchak and Zachor [34] also sought to characterize the stability and changes of autism diagnosis in correlation with pretreatment predictors and post-intervention outcomes. The open-design study investigated a sample size of 68 (62 boys), aged 18 to 35 mon. Inclusion criteria required that the child met established DSM-IV criteria for autism. Exclusion criteria were comorbidities, including genetic syndromes and seizure disorders. The main outcomes of this experiment suggest that individuals who had a changed diagnostic classification to ASD or Off Spectrum had better receptive language scores, as well as significant improvements in cognitive outcomes, adaptive outcomes, and reduction of stereotyped behaviors, as compared to individuals within the unchanged classification group.

Kasari, Paparella, Freeman, and Jahromi [35] investigated the effects of joint attention (JA) and symbolic play (SP) behavioral interventions in accordance with prediction to language outcomes. The study analyzed a sample size of 46 boys, aged 36 to 48 mon. Inclusion criteria required that the children had been diagnosed with autism on the ADI-R and ADOS scale, had to be of 5 years of age or younger, and had to be accessible for follow-ups. Exclusion criteria included seizure disorder and additional medical diagnoses, such as genetic syndromes. The main outcomes of this experiment included greater JA and SP skills and ability to execute these skills during play, within the respective groups as compared to the control group.

Ben-Itzchak and Zachor [36] sought to understand the correlation between cognitive, socialization, and communication pre-intervention variables to outcome in children with autism post-intervention. The study investigated a sample size of 25 (23 boys), aged 20 to 32 mon. Inclusion criteria included children diagnosed using the ADI-R and ADOS protocols. Exclusion criteria included children who demonstrated comorbidities, including genetic syndromes and seizure disorders. The main outcomes of this experiment were that the children demonstrated significant improvements in imitation, receptive and expressive language, nonverbal communication, play skills, and stereotyped behaviors.

Remington et al. [37] investigated the effects of early intensive behavioral intervention for children with autism. The quasi-experiment analyzed a sample size of 44, aged 30 to 42 mon. Exact numbers of male and female participants were not given. Inclusion criteria included that the children had to be diagnosed with autism based on the ADI-R, had a previous diagnosis of autism by a clinician independent of the research program, or had a suspected diagnosis of autism, to be between 30 and 42 mon of age at the time of induction, and had to live in their family home. The exclusion criteria included that the child had to be free of any other chronic or serious medical conditions that might interfere with the ability to deliver consistent intervention or might adversely affect development. The main outcomes included significant improvements in IQ scores, daily living skills, motor skills, and language abilities subsequent to the interventional therapies. Moreover, children who participated in the early behavioral intervention therapy were more likely to attend mainstream schools, as compared to children within the control group.

Zachor, Ben-Itzchak, Rabinovich, and Lahat [38] compared the Eclectic-Development (ED) and ABA intervention approaches in children with autism. The quasi-experiment analyzed a sample size of 39 (37 boys), aged 22 to 34 mon. Inclusion criteria included that the children were diagnosed with autism using the ADI, met established criteria for Autism/PDD-NOS according to DSM-IV criteria. Exclusion criteria included children who had medical abnormalities such as seizures or hearing deficiencies. The main outcomes of this experiment demonstrated that ABA intervention approaches provided children with greater improvements in language communication and social interaction, as well as allowed for greater changes in diagnostic classifications, as compared to ED intervention approaches.

Cohen, Amerine-Dickens, and Smith [39] sought to investigate the effects of early intensive behavioral treatment (EIBT) for children with autism. The quasi-experiment utilized a sample of 42 (35 boys), aged 20 to 41 mon. Inclusion criteria included that children had a primary, previous, and psychological diagnosis of autistic disorder or pervasive development disorder confirmed by ADI-R, pretreatment IQ above 35 on the Bayley Scales of Infant Development-Revised (BSID-R), chronological

age between 18 and 42 mon at diagnosis and under 48 mon at treatment onset, residence within 60 kilometers of the treatment agency, and parental agreement to active participation. Exclusion criteria included children who had a severe medical limitation or illness, including motor or sensory deficits, that would prevent a child from participating in treatment for 30 h a week, and children who had undergone more than 400 h of prior behavioral intervention. The main outcomes of this experiment suggested a significant difference in the IQ scores and adaptive behavior for children who had undergone the EIBT, and a significant increase in EIBT children in regular education as compared to the control group. However, there were no significant between-group differences in language comprehension or nonverbal skills.

Kasari, Freeman, and Paparella [40] examined the efficacy of JA- and SP-targeted interventions. The randomized controlled study investigated a sample size of 58 (46 boys), aged 36 to 48 mon. Inclusion criteria included that children had a diagnosis of autism on the ADI-R and ADOS, were of 5 years of age or younger, and were accessible for follow-ups. Exclusion criteria included no seizure disorders or additional medical diagnoses, and children whose parents demonstrated refusal of final assessments or who left the program unexpectedly. The main outcomes of this experiment demonstrated improvements of JA and SP within the respective experimental groups, as well as significantly greater growth in expressive language for the individuals within these groups.

Eikeseth, Hayward, Gale, Gitlesen, and Eldevik [41] investigated the outcomes of varying intensities of early behavioral intervention for children with autism. The open-design study initially analyzed a sample size of 23 (17 boys), aged 28 to 42 mon. Inclusion criteria included diagnosis of autism according to the ICD-10 (International Classification of Diseases), chronological age at intake between 24 and 42 mon, the absence of other severe medical conditions as certified by a medical practitioner, and if the child resided outside of the catchment area for the clinical-based services. Exclusion criteria included an increased intensity of supervision due to lack of acquisition (as was the case for one child). The main outcomes of this experiment demonstrated a correlation between the intensity of supervision with changes in IQ scores and visual-spatial IQ after 14 mon. However, there was no significant correlation with the intensity of supervision and adaptive functioning.

Many of the studies that fell within the restrictive exclusion criteria category demonstrated positive outcomes of early behavioral interventions on various developmental skills including autism severity, verbal communication, social interaction, and other markers of development in comparison to control groups. Thus, these studies demonstrated promising results in improvement of many skills for young children with ASD. However, the restrictive nature of these studies limits the applicability of their outcomes to a wider audience of children with ASD who present with some form of comorbidity.

### *3.2. Loosely Defined Exclusion Criteria*

Of the studies discussed in this review, 15% ($n$ = 4/26) utilized loosely defined exclusion criteria for their early-intervention behavioral treatments. Studies with loosely defined criteria included children who experienced ASD with comorbidities but excluded subjects based on other factors, such as primary language and accessibility to testing sites, or severe motor or sensory deficits.

Yoder and Stone [42] evaluated two different communication interventions: Responsive Education and Prelinguistic Milieu Teaching (RPMT) and the Picture Exchange Communication System (PECS) in preschool children with ASD. The randomized group experiment included 36 children with a diagnosis of ASD or PDD-NOS aged 18 to 60 mon, who demonstrated communication deficits and passed hearing screenings. Of the participating children, 31 were boys. Participants were excluded from the study if they demonstrated severe sensory or motor deficits or if English was not the primary language spoken in the home. Of the 120 children who were screened for participation in the study, only 60 met inclusion criteria. Results demonstrated mixed results, with RPMT demonstrating better effects with generalized turn taking and generalized joint attention initiation as compared to PECS. Conversely, PECS demonstrated better effects with generalized requests in children who arrived to the study with little initiation of joint attention.

Oosterling et al. [43] strove to understand the efficacy of non-intensive parental training in combination with standard care for children with autism. The randomized, controlled trial investigated a sample size of 75 (52 boys), aged 12 to 24 mon. Inclusion criteria included children with a clinical diagnosis of ASD or PDD-NOS, a demonstrated developmental potential at 12 mon, and a developmental quotient below 80. Exclusion criteria included family problems that may interfere with parental training and insufficient parental proficiency in the native language, Dutch. The main outcomes of this experiment suggested that additional non-intensive parental training did not have any influence on language and global clinical improvement outcome variables.

Wetherby et al. [44] sought to compare the effects of two parent-implemented Early Social Interaction (ESI) interventions. The randomized, controlled trial investigated a sample size of 82, aged 16 to 20 mon. Exact numbers of male and female participants were not given, but the individual ESI group contained 81% male participants, and the group ESI contained 92.5% male participants. Inclusion criteria included children who had received an ASD diagnosis between ages 16 to 20 mon and lived within 50 miles of either research site. Exclusion criteria included children who demonstrated participation in other interventional research studies. The main outcomes demonstrated that children within the individual social intervention groups improved their social communication, daily living, receptive language, and social skills, while children within the group intervention groups demonstrated worsening or no significant change in these measures.

Howard, Sparkman, Cohen, Green, and Stanislaw [45] compared the effects of intensive behavior analytic intervention (IBT), intensive eclectic intervention, and non-intensive public early-intervention programs in children with autism. The quasi-experiment investigated a sample size of 61 (54 boys), all less than 48 mon of age. Inclusion criteria included children who were independently diagnosed with Autistic Disorder or PDD-NOS according to DSM-IV criteria, entry into an intervention program before 48 mon of age, English spoken as the primary language within the child's home, no significant and separate medical condition, and no prior treatment of more than 100 h. Exclusion criteria included individuals who had not completed the 7 mon of intervention, and parents who could not be contacted to arrange follow-up testing despite repeated attempts or refusal of testing. The main outcomes of this trial demonstrated that individuals who participated in the IBT group performed significantly higher in tests for IQ, nonverbal and verbal language, overall communication, and social skills.

The studies that utilized loosely defined exclusion criteria provide a stronger foundation to apply certain early-intervention behavioral methods to a wider range of children with ASD, given that they included a more diverse participant pool. However, not only are there a limited number of studies available with this type of exclusion criteria, but the criteria were often so specific to the particular study that it inhibited any potential conclusions that may be drawn in understanding the applicability of these outcomes to a wider range of children with ASD. This could compromise the generalizability of the results of these studies to a wide range of children with ASD.

### 3.3. Exclusion Criteria Not Defined

Of the studies discussed in this review, 30% ($n$ = 7/26) did not specifically list any exclusion criteria for the participants of their early-intervention behavioral treatments, and thus, the results of these studies may be applied to the comparably widest range of children with ASD.

Welterlin, Turner-Brown, Harris, Mezibov, and Delmolino [46] implemented the Treatment and Education of Autistic and Communication Related handicapped Children (TEACCH) program in home-based models for parents of toddlers with ASD. Inclusion criteria for the study were chronological age of less than 42 mon and a diagnosis of Autism. No other exclusion criteria were specified. Twenty children participated in the study and were randomly assigned to receive TEACCH intervention at home or wait-list control. Six children participated in the experimental group and, of these, five were male. Participants were matched for data analysis between the experimental and control groups on the basis of similar age. Results between the experimental and control group did not reach statistical significance, which the authors attributed to low sample size and short time frame.

Reed, Osborne, and Corness [47] conducted a study of 33 children who were nonrandomly assigned to treatment groups. Inclusion criteria were as follows: Age of 2 years, 6 mon to 4 years, 0 mon at the start of their intervention, and a diagnosis of ASD. No details were given about the number of males and females that participated. The only exclusion criterion specified was that the children participating in the study must not have been involved in any other major intervention at the same time as the study. Children were divided into one of three treatment groups. One group received preschool special education, another received special education designed specifically for autism, and the final group received in-home one-on-one behavioral treatment. After 10 mon of intervention, results from the three groups were compared, with some improvement in measures used across both special education groups. Children in the home-based program showed improvement across the Psych-Educational Profile and British Abilities Scale, but not for the Vineland Adaptive Behavior Scales.

Smith, Flanagan, Garon, and Bryson [48] examined Pivotal Response Training (PRT) in an Early Intensive Behavioral Intervention (EIBI) program delivered in the community. Inclusion criteria for the study were: Having a diagnosis of Autism Spectrum Disorder and age below 6 years. Children who met eligibility criteria were randomly assigned to participate in the experimental group. No control group was used. Rather, participants were divided into subgroups for data analysis, based on their scores on measures of intellectual functioning. Results demonstrated that all study participants, regardless of cognitive functioning level, showed significant improvement in communication skills and adaptive functioning, with larger gains found for the children in the moderate and high cognitive functioning groups.

Fernell et al. [49] conducted a naturalistic, prospective study with 208 children aged $1\frac{1}{2}$ to $4\frac{1}{2}$ years. No information was given on the number of males and females included in the study. Children included in the study had a previous diagnosis of Autism that was confirmed through further testing for inclusion in the study, but no exclusion criteria were given, beyond parents' language proficiency in Swedish or English. All children in the study received some form of applied behavior analysis (ABA), and participants self-selected into intensive ABA or non-intensive ABA. There was no control group. This study showed that study participants improved in several areas of functioning, and participants in intensive intervention did not show more improvement than participants in non-intensive intervention.

Landa, Holman, O'Neill, and Stuart [50] evaluated the effects of a curriculum aimed to improve socially synchronous behaviors for children with autism. The randomized, controlled trial investigated a sample size of 48 (40 boys), aged 21 to 23 mon. The inclusion criteria specified that the children met criteria on the ADOS, received a diagnosis of ASD from an expert clinician, had a nonverbal mental age of at least 8 mon, had no siblings with ASD, English was the primary language spoken within the home, and no known etiology for ASD. No exclusion criteria were specifically listed. The main outcomes for this experiment included significant between-group differences for socially engaged imitation, but no significant between-group differences for shared positive affect, expressive language, or nonverbal cognition.

Ingersoll [51] evaluated the efficacy of Reciprocal Imitation Training (RIT) in development elicited and spontaneous imitation skills in children with autism. The randomized, controlled trial investigated a sample size of 21 (18 boys), aged 27 to 47 mon. The inclusion criteria mandated that the children receive a clinical diagnosis of autism based on DSM-IV-TR criteria and met the cut-off for ASD on ADOS. There were no exclusion criteria that were explicitly listed. The main outcomes for the experiment included significantly more gains in elicited and spontaneous imitation for both objects and gestures, as compared to the control.

Reed, Osborne, and Corness [52] investigated the efficacy of home-based early behavioral interventions for children with autism. The quasi-experiment investigated a sample size of 27 (27 boys), aged 31 to 48 mon. Children included in the study were within 2 years, 6 mon and 4 years of age, received no other major intervention during the period of assessment, and had a diagnosis of ASD. The exclusion criteria were not listed. The main outcomes of this experiment demonstrated significant

between-group differences in educational functioning, with no significant between-group differences for intellectual functioning, adaptive behavior, and ASD severity.

The studies described above that did not specifically exclude children from participating in the study present results that are generalizable to the broadest population of children with ASD. However, this same lack of any exclusionary criteria also prevents understanding the specific methods of treatment necessary for the many different types of children who are diagnosed with ASD. Thus, the wider generalizability leads to fewer conclusions that can be drawn about the applicability of these results to any one specific child.

## 4. Discussion

This review evaluated 26 early-intervention behavioral studies of ASD based on their exclusion criteria into three categories: Restrictive, loosely defined, and not defined. These categories carry critical implications, as these categories define which of their outcomes may be applied to various audiences of children with ASD.

There were 15 studies that utilized restrictive criteria risk excluding approximately 75% of children who have ASD with a comorbid condition, including the 10% with a co-occurring psychiatric disorder, and the 4% with a genetic or chromosomal disorder [53]. Others excluded children with common neurological conditions, such as fragile X syndrome or epilepsy, which are strongly associated with autism [25]. Prevalence of ASD in children with epilepsy is around 6.3% with higher prevalence up to 47% in children with other forms of seizure disorders [54]. Other studies excluded children born before 35 weeks, although some studies suggest that about 7% of preterm infants might develop autism [55]. These studies may exclude a large group of individuals with ASD. Although many of these studies categorized the children as having improved, the results suggest that interventions work only for the minority of children who have "pure" ASD. For this reason, it is not possible to conclude that early intervention works in all children with ASD.

The four studies that utilized loosely defined exclusion criteria and the seven studies that did not define any exclusion criteria may have included children with comorbid disorders that could have influenced their findings. Indeed, these studies showed mixed results, with some experimental groups showing more improvement than control groups, and others showing no significant between-group differences. Inclusion of comorbidities makes these studies' results more applicable to a wide range of children with ASD, but also makes it difficult to know which interventions might be efficacious for specific comorbidities with ASD, since inclusion of comorbidities was typically not limited to only specific disorders.

We believe that studies that investigate behavioral interventions for young children with ASD should make more of an effort to recruit and include study participants with comorbid conditions in addition to ASD, which could make their results more applicable to a wider range of children with ASD. It will also be important for these comorbid conditions to be explicitly listed in the participant characteristics so that conclusions can be drawn about how efficacious certain behavioral interventions are for children with ASD and associated conditions. Listing the participants with these descriptors may make it easier to understand what population of children with ASD may be most likely to benefit from the interventions studied.

Current guidelines suggest not to exclude individuals with associated conditions if these are common. Given the number and incidence of comorbid disorders it may be hard to try to identify individuals who only meet criteria for ASD and no other disorders. Moreover, this may not be representative of the population of children with ASD. This review highlights the possible influence of treatment modifiers such as comorbidity in the outcome of behavioral interventions for young children with ASD. Overall, the results suggest that the heterogeneity observed in the response to early behavioral intervention in children with ASD may be related to various comorbid conditions. They underscore the need to systematically screen for the presence of comorbid symptoms and conditions at the time of recruitment of subjects, identify these in their studies, and modify intervention

methods accordingly. How those interventions should be modified remains unclear as there is not yet enough research evidence to suggest what are evidence-based interventions for ASD with comorbid conditions.

A supplementary table, depicting the studies included in this review, grouped by intervention type, is available in Table A2.

## 5. Limitations

There are some important limitations in this literature review. To begin, this review only included studies that used a group design. This is an important limitation about the results of this review, given that many studies investigating a behavioral intervention for young children with ASD use single-subject research design [56], which has been increasing over recent years [57]. However, group study designs for investigating behavioral interventions for individuals with ASD are an important part of identifying evidence-based practices for ASD [58] and allow for decisions to be made about the efficacy of a particular intervention [57]. In addition, the research databases used (PsychINFO, PubMed) are widely used and represent many research studies, but they are not inclusive of all research being conducted, so it is possible that some studies that could have met this review's inclusion criteria were missed.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A

**Table A1.** Summary of early-intervention studies reviewed.

| Restrictive Exclusion Criteria<br>studies that excluded children with co-morbidities and/or associated family mental health conditions | | | | |
|---|---|---|---|---|
| **Authors** | **Sample** | **Inclusion Criteria** | **Exclusion Criteria** | **Main Outcomes** |
| Perera, Jeewandara, Seneviratne, & Guruge (2016) [28] | 62 children (48 boys) aged 18–40 mon | Children aged 18 to 40 mon and who were diagnosed with autism for the first time at intake and had not received developmental interventions of any form previously. | Exclusions: (i) those diagnosed with other pervasive developmental disorders and Asperger disorder, (ii) those with severe cognitive impairment with autistic features, (iii) those diagnosed with autism having associated motor and sensory disorders and genetic disorders, (iv) those who had received other developmental interventions before intake and during the course of the study, and (v) those who dropped out before completion of the intervention period. | Children in the experimental group showed more improvement on measures of autism severity and social interaction, despite some improvement in the children in the comparison group |
| Brian, Smith, Zwaigenbaum& Bryson (2017) [29] | 62 children aged 16–30 mon | Children with either confirmed ASD diagnosis or elevated scores on measures that assess ASD symptoms, no more than half-time childcare, between 36 and 42 weeks' gestation, birthweight >2500 g, and absence of identifiable neurological, genetic, or severe sensory or motor conditions | Not specifically listed, but 11 children were evaluated and determined not to fit study inclusion criteria, and additional child met inclusion criteria but dropped out of the study early and those results were not included in the analysis | Children in the experimental group showed significant gains over the control group in the following areas assessed: child functional vocal responsiveness to parent prompts, child vocal initiations, parent smiling, fidelity of implementation, and parent-reported self-efficacy |
| Rogers et al. (2012) [30] | 98 children (76 boys) aged 12–24 mon | Children met risk criteria for ASD in a clinical assessment, were ambulatory, had a development quotient of 35 or higher, and primarily spoke English within the home | Children who had parents that self-reported mental illness or substance abuse, children who had significant medical conditions such as cerebral palsy, a gestational age of less than 35 weeks, genetic disorders related to developmental disabilities, or individuals who had current or prior enrollment in an intensive 1:1 autism intervention curriculum for more than 10 h per week | Individuals who had received parental training in the Early Start Denver Model technique established more productive working alliances with their therapists as compared to the community group, however, the effects seen in intensive-treatment studies were not observed. Younger age and greater intervention h positively affected the developmental rates for children with autism |

**Table A1.** *Cont.*

| Restrictive Exclusion Criteria<br>studies that excluded children with co-morbidities and/or associated family mental health conditions | | | | |
|---|---|---|---|---|
| **Authors** | **Sample** | **Inclusion Criteria** | **Exclusion Criteria** | **Main Outcomes** |
| Carter et al. (2011) [31] | 62 children (51 boys) aged 15–25 mon | Children that met the criteria of being diagnosed with ASD, and were recruited from ASD specialty clinics | Children that had a genetic disorder, children who did not obtain a pre-determined "at-risk" score on the Screening Tool for Children with Autism (STAT), or children who did meet the symptom criteria for an ASD diagnosis based on clinical evaluations | The intervention model, HMTW, showed differential effects on child communication based on a baseline factor, but parents of children who possessed higher object interest may require additional support to implement the proper strategies |
| Dawson et al. (2010) [12] | 48 children (3.5M:1F) aged 18–30 mon | Children must meet criteria for ASD on the Toddler Autism Diagnostic Interview and ADOS, receive a clinical diagnosis for ASD based on DSM-IV criteria, had to reside within half an hour of the testing location, and demonstrate a willingness to participate in a 2-year or greater intervention | Children who had a neurodevelopmental disorder of known etiology, significant sensory or motor impairments, major physical problems such as chronic or serious health conditions, seizures at the time of entry, use of psychoactive medication, history of serious head injury or neurological disease, alcohol or drug exposure during the prenatal period, or developmental quotient below 35 | ESDM group demonstrated significant improvements in IQ and adaptive behavior and were more likely to have a change in diagnosis to PDD-NOS. Comparison group manifested greater delays in adaptive behaviors and demonstrated minimal improvement in baseline scores |
| Kasari, Gulsrud, Wong, Kwon, & Locke (2010) [32] | 38 children (29 boys) aged 21–36 mon | Children must have met criteria for autism following DSM-IV criteria by an independent clinician | Children with additional syndromes | Experimental group made significant improvements in joint engagement, responsiveness and diversity of functional play acts, as compared to the control group |
| Zachor & Itzchak (2010) [33] | 78 children (71 boys) aged 15–35 mon | Participating children had to meet a clinical diagnosis of autism based on DSM-IV criteria and the cut-off points on the ADI-R | Additional major medical diagnoses or incomplete post-intervention assessments | No significant between-group differences in improved cognitive abilities or adaptive skills; Group with less severe baseline ASD and received eclectic intervention had better outcomes in communication, socialization, and adaptive skills |

**Table A1.** *Cont.*

| Restrictive Exclusion Criteria<br>studies that excluded children with co-morbidities and/or associated family mental health conditions | | | | |
|---|---|---|---|---|
| **Authors** | **Sample** | **Inclusion Criteria** | **Exclusion Criteria** | **Main Outcomes** |
| Itzchak & Zachor (2009) [34] | 68 children (62 boys) aged 18–35 mon | Child met established DSM-IV criteria for autism | Comorbidities, including genetic syndromes and seizure disorders | Group with changed diagnostic classification had better receptive language scores, significant improvements in cognitive and adaptive outcomes, reduction of stereotyped behaviors |
| Kasari, Paparella, Freeman, & Jahromi (2008) [35] | 46 boys aged 36–48 mon | Children had been diagnosed with autism on the ADI-R and ADOS scale, 5 years of age or younger, and be accessible for follow-ups | Seizure disorder and additional medical diagnoses, such as genetic syndromes | Greater JA and SP skills, and ability to execute these skills during play, as compared to the control group |
| Ben-Itzchak & Zachor (2007) [36] | 25 children (23 boys) aged 20–32 mon | Children diagnosed using the ADI-R and ADOS protocols | Children who demonstrated comorbidities, including genetic syndromes and seizure disorders | Children demonstrated significant improvements in imitation, receptive and expressive language, nonverbal communication, play skills, and stereotyped behaviors |
| Remington et al. (2007) [37] | 44 children aged 30–42 mon | Diagnosed with autism based on the ADI-R, or a previous diagnosis of autism by a clinician independent of the research program, or suspected diagnosis of autism, between 30 and 42 mon of age, and live in their family home | Free of any other chronic or serious medical conditions that might interfere with the ability to deliver consistent intervention or might adversely affect development | Significant improvements in IQ, daily living skills, motor skills, and language abilities. Early behavioral intervention group more likely to attend mainstream schools, compared to control group |
| Zachor, Ben-Itzchak, Rabinovich, & Lahat (2007) [38] | 39 children (37 boys) aged 22–34 mon | Children were diagnosed with autism using the ADI, met established criteria for autism/PDD-NOS according to DSM-IV criteria | Children that had medical abnormalities such as seizures of hearing deficiencies | ABA intervention group had greater improvements in language and social interaction greater changes in diagnostic classifications, as compared to ED intervention approaches |
| Cohen, Amerine-Dickens, & Smith (2006) [39] | 42 children (35 boys) aged 20–41 mon | Previous diagnosis of autistic disorder or PDD-NOS confirmed by ADI-R, IQ above 35 on the BSID-R, chronological age between 18-42 mon at diagnosis and under 48 mon at treatment onset, residence within 60 kilometers of the treatment agency | Children that had a severe medical limitation or illness, including motor or sensory deficits, that would prevent a child from participating in treatment for 30 h a week, and children that had underwent more than 400 h of prior behavioral intervention | EIBT group had significant difference in IQ and adaptive behavior and a significant increase in attendance in regular education compared to the control group. No significant between-group differences in language comprehension or nonverbal skills |

**Table A1.** *Cont.*

| Authors | Sample | Inclusion Criteria | Exclusion Criteria | Main Outcomes |
|---|---|---|---|---|
| **Restrictive Exclusion Criteria**<br>studies that excluded children with co-morbidities and/or associated family mental health conditions | | | | |
| Kasari, Freeman, & Paparella (2006) [40] | 58 children (46 boys) aged 36–48 mon | Children had a diagnosis of autism on the ADI-R and ADOS, were of 5 years of age or younger, and were accessible for follow-ups | No seizure disorders or additional medical diagnoses, and children whose parents demonstrated refusal of final assessments or who left the program unexpectedly | Improvements of JA and SP within the respective experimental groups, as well as significantly greater growth in expressive language for the individuals within these groups |
| Eikeseth, Hayward, Gale, Gitlesen, & Eldevik (2009) [41] | 23 children (17 boys) aged 28–42 mon | Diagnosis of autism according to the ICD-10, chronological age between 24 and 42 mon, the absence of other severe medical conditions, and if the child resided outside of the catchment area for the clinical-based services | Included an increased intensity of supervision due to lack of acquisition (as was the case for one child) | Demonstrated a correlation between the intensity of supervision with changes in IQ and visual-spatial IQ after 14 mon. However, there was no significant correlation with the intensity of supervision and adaptive functioning |
| Loosely Defined Exclusion Criteria<br>studies that included children with comorbidities but excluded on the basis of other non-diagnostic factors | | | | |
| Yoder & Stone (2006) [42] | 36 children (31 boys) aged 18–60 mon | A diagnosis of autistic disorder or PDD-NOS; chronological age of 18 to 60 mon; fewer than 10 words in communication samples; and passed hearing screenings | Child were excluded who demonstrated severe sensory or motor deficits or if English was not the primary language spoken in the home | RMPT group showed higher frequency of generalized turn taking and generalized initiating joint attention. PECS facilitated generalized requests more than the RPMT in children with very little initiating joint attention prior to treatment. |
| Oosterling et al. (2010) [43] | 75 children (52 boys) aged 12–24 mon | Clinical diagnosis of ASD or PDD-NOS, and a demonstrated developmental potential at 12 mon, and a developmental quotient below 80 | Family problems that may interfere with parental training and insufficient parental proficiency in the native language, Dutch | Additional non-intensive parental training did not have any influence on language and global clinical improvement outcome variables |
| Wetherby et al. (2014) [44] | 82 children aged 16–20 mon | Received ASD diagnosis between ages 16 to 20 mon and lived within 50 miles of either research site | Participation in other interventional research studies | Individual intervention improved social communication, daily living, receptive language, and social skills, while group intervention participants demonstrated worsening or no significant changes |
| Howard, Sparkman, Cohen, Green, & Stanislaw (2005) [45] | 61 children (54 boys) less than 48 mon of age | Independently diagnosed with autistic disorder or PDD-NOS according to DSM-IV, entry into an intervention program before 48 mon of age, English spoken as the primary language within the child's home, no significant and separate medical condition, and no prior treatment of more than 100 h | Individuals who had not completed the 7 mon of intervention, and parents who could not be contacted to arrange follow-up testing despite repeated attempts or refusal of testing | Individuals who participated in the IBT group performed significantly higher in tests for IQ, nonverbal and verbal language, overall communication, and social skills |

**Table A1.** *Cont.*

| Restrictive Exclusion Criteria<br>studies that excluded children with co-morbidities and/or associated family mental health conditions | | | | |
|---|---|---|---|---|
| **Authors** | **Sample** | **Inclusion Criteria** | **Exclusion Criteria** | **Main Outcomes** |
| Exclusion Criteria Not Defined<br>studies that have not significantly excluded any children | | | | |
| Welterlin, Turner-Brown, Harris, Mezibov, & Delmolino (2012) [46] | 20 children, 2–3 years, 5 males in experimental group | Chronological age of less than 42 mon and a clinical diagnosis of autism | None | Mixed results; Treatment group showed improvements in independent work skills and parent ability to structure environment for learning; but no between groups differences could be supported for developmental gains or parent stress |
| Reed, Osborne, & Corness, 2010 [47] | 33 children aged 2.5 to 4 years | Children included were aged 2:6 to 4:0 years at the start of their intervention; receiving no other major intervention during the period of the assessment; and had to have a diagnosis of ASD given by an independent pediatrician | None | Moderate improvements for children in all 3 groups. |
| Smith, Flanagan, Garon, & Bryson (2015) [48] | 118 children aged 2–5 years (86% boys) | Children were selected randomly for the intervention program by their ASD diagnosis and age below 6 years | None | Significant gains in key language and cognitive outcomes for all groups. Baseline cognitive scores significantly predicted 1-year outcomes |
| Fernell et al. (2011) [49] | 208 children aged 20–54 mon | Children had existing ASD diagnoses, no other inclusion criteria specified | None | Vineland composite scores increased over the 2-year period for by the subgroup with normal cognitive functioning. There was no significant difference between the intensive and non-intensive groups |
| Landa, Holman, O'Neill, & Stuart (2010) [50] | 48 children (40 boys) aged 21–23 mon | Children met criteria on ADOS for ASD, Diagnosis of ASD from an expert clinician, had a non-verbal mental age of at least 8 mon, had no siblings with ASD, English the primary language spoken, and no known etiology for ASD | None | Significant between-group differences for socially engaged imitation, but no significant between-group differences for shared positive affect, expressive language, or nonverbal cognition |
| Ingersoll (2010) [51] | 21 children (18 boys) aged 27–47 mon | Children receive a clinical diagnosis of autism based on DSM-IV-TR criteria and met the cut-off for ASD on ADOS | None | Significantly more gains in elicited and spontaneous imitation for both objects and gestures, as compared to the control |
| Reed, Osborne, & Corness (2007) [52] | 27 children (27 boys) aged 31–48 mon | Children were within 2 years, 6 mon and 4 years of age, received no other major intervention during the period of assessment, and had a diagnosis of ASD | None | Significant between-group differences in educational functioning, with no significant between-group differences for intellectual functioning, adaptive behavior, and ASD severity |

**Table A2.** Studies reviewed grouped by intervention type.

| **Interventions based on Applied Behavior Analysis**<br>studies that investigated interventions based on the principles of ABA, such as early intensive behavioral intervention, TEACCH | | | | |
|---|---|---|---|---|
| **Authors** | **Sample** | **Exclusion Criteria Category** | **Intervention Investigated** | **Main Outcomes** |
| Zachor & Itzchak (2010) [33] | 78 children (71 boys) aged 15–35 mon | Restrictive | Applied behavior analysis | No significant between-group differences in improved cognitive abilities or adaptive skills; Group with less severe baseline ASD and received eclectic intervention had better outcomes in communication, socialization, and adaptive skills |
| Itzchak & Zachor (2009) [34] | 68 children (62 boys) aged 18–35 mon | Restrictive | Early intensive behavioral intervention (EIBI) vs. eclectic therapies | Group with changed diagnostic classification had better receptive language scores, significant improvements in cognitive and adaptive outcomes, reduction of stereotyped behaviors |
| Ben-Itzchak & Zachor (2007) [36] | 25 children (23 boys) aged 20–32 mon | Restrictive | Early intensive behavioral intervention (EIBI) | Children demonstrated significant improvements in imitation, receptive and expressive language, nonverbal communication, play skills, and stereotyped behaviors |
| Remington et al. (2007) [37] | 44 children aged 30–42 mon | Restrictive | Early intensive behavioral intervention (EIBI) | Significant improvements in IQ, daily living skills, motor skills, and language abilities. Early behavioral intervention group more likely to attend mainstream schools, compared to control group |
| Zachor, Ben-Itzchak, Rabinovich, & Lahat (2007) [38] | 39 children (37 boys) aged 22–34 mon | Restrictive | ABA intervention vs. eclectic therapies | ABA intervention group had greater improvements in language and social interaction greater changes in diagnostic classifications, as compared to ED intervention approaches |
| Cohen, Amerine-Dickens, & Smith (2006) [39] | 42 children (35 boys) aged 20–41 mon | Restrictive | Early intensive behavioral intervention (EIBI) | EIBT group had significant difference in IQ and adaptive behavior and a significant increase in attendance in regular education compared to the control group. No significant between-group differences in language comprehension or nonverbal skills |

**Table A2.** *Cont.*

| Interventions based on Applied Behavior Analysis<br>studies that investigated interventions based on the principles of ABA, such as early intensive behavioral intervention, TEACCH | | | | |
|---|---|---|---|---|
| **Authors** | **Sample** | **Exclusion Criteria Category** | **Intervention Investigated** | **Main Outcomes** |
| Eikeseth, Hayward, Gale, Gitlesen, & Eldevik (2009) [41] | 23 children (17 boys) aged 28–42 mon | Restrictive | Early intensive behavioral intervention (EIBI) | Demonstrated a correlation between the intensity of supervision with changes in IQ and visual-spatial IQ after 14 mon. However, there was no significant correlation with the intensity of supervision and adaptive functioning |
| Howard, Sparkman, Cohen, Green, & Stanislaw (2005) [45] | 61 children (54 boys) less than 48 mon of age | Loosely-defined | Early intensive behavioral intervention (EIBI) vs. eclectic therapies | Individuals who participated in the IBT group performed significantly higher in tests for IQ, nonverbal and verbal language, overall communication, and social skills |
| Welterlin, Turner-Brown, Harris, Mezibov, & Delmolino (2012) [46] | 20 children, 2–3 years, 5 males in experimental group | Not defined | TEACCH | Mixed results; Treatment group showed improvements in independent work skills and parent ability to structure environment for learning; but no between groups differences could be supported for developmental gains or parent stress |
| Reed, Osborne, & Corness, 2010 [47] | 33 children aged 2.5 to 4 years | Not defined | ABA therapy vs. normal educational practice | Moderate improvements for children in all 3 groups. |
| Smith, Flanagan, Garon, & Bryson (2015) [48] | 118 children aged 2–5 years (86% boys) | Not defined | Pivotal Response Training | Significant gains in key language and cognitive outcomes for all groups. Baseline cognitive scores significantly predicted 1-year outcomes. |
| Fernell et al. (2011) [49] | 208 children aged 20–54 mon | Not defined | Early intensive behavioral intervention (EIBI) | Vineland composite scores increased over the 2-year period for by the subgroup with normal cognitive functioning. There was no significant difference between the intensive and non-intensive groups |
| Reed, Osborne, & Corness (2007) [52] | 27 children (27 boys) aged 31–48 mon | Not defined | Early intensive behavioral intervention (EIBI) | Significant between-group differences in educational functioning, with no significant between-group differences for intellectual functioning, adaptive behavior, and ASD severity |

**Table A2.** *Cont.*

| Authors | Sample | Exclusion Criteria Category | Intervention Investigated | Main Outcomes |
|---|---|---|---|---|
| **Interventions based on Applied Behavior Analysis** studies that investigated interventions based on the principles of ABA, such as early intensive behavioral intervention, TEACCH | | | | |
| **Early Start Denver Model** | | | | |
| Rogers et al. (2012) [30] | 98 children (76 boys) aged 12–24 mon | Restrictive | ESDM | Individuals who had received parental training in the Early Start Denver Model technique established more productive working alliances with their therapists as compared to the community group, however, the effects seen in intensive-treatment studies were not observed. Younger age and greater intervention h positively affected the developmental rates for children with autism |
| Dawson et al. (2010) [12] | 48 children (3.5M:1F) aged 18–30 mon | Restrictive | ESDM | ESDM group demonstrated significant improvements in IQ and adaptive behavior and were more likely to have a change in diagnosis to PDD-NOS. Comparison group manifested greater delays in adaptive behaviors and demonstrated minimal improvement in baseline scores |
| **Joint Attention and Symbolic Play Interventions** | | | | |
| Kasari, Gulsrud, Wong, Kwon, & Locke (2010) [32] | 38 children (29 boys) aged 21–36 mon | Restrictive | Joint attention intervention | Experimental group made significant improvements in joint engagement, responsiveness and diversity of functional play acts, as compared to the control group |
| Kasari, Paparella, Freeman, & Jahromi (2008) [35] | 46 boys aged 36–48 mon | Restrictive | Joint attention and symbolic play | Greater JA and SP skills, and ability to execute these skills during play, as compared to the control group |
| Kasari, Freeman, & Paparella (2006) [40] | 58 children (46 boys) aged 36–48 mon | Restrictive | Joint attention and symbolic play | Improvements of JA and SP within the respective experimental groups, as well as significantly greater growth in expressive language for the individuals within these groups |
| **Interventions Primarily Targeting Speech and Language** | | | | |
| Carter et al. (2011) [31] | 62 children (51 boys) aged 15–25 mon | Restrictive | Hanen's More Than Words | The intervention model, HMTW, showed differential effects on child communication based on a baseline factor, but parents of children who possessed higher object interest may require additional support to implement the proper strategies |

**Table A2.** *Cont.*

| Authors | Sample | Exclusion Criteria Category | Intervention Investigated | Main Outcomes |
|---|---|---|---|---|
| **Interventions based on Applied Behavior Analysis**<br>studies that investigated interventions based on the principles of ABA, such as early intensive behavioral intervention, TEACCH | | | | |
| **Interventions Primarily Targeting Speech and Language** | | | | |
| Yoder & Stone (2006) [42] | 36 children (31 boys) aged 18–60 mon | Loosely-defined | Responsive Education and Prelinguistic Milieu Training (RPMT) and Picture Exchange Communication System (PECS) | RMPT group showed higher frequency of generalized turn taking and generalized initiating joint attention. PECS facilitated generalized requests more than the RPMT in children with very little initiating joint attention prior to treatment. |
| **Parent-Mediated Behavioral Interventions** | | | | |
| Perera, Jeewandara, Seneviratne, & Guruge (2016) [28] | 62 children (48 boys) aged 18–40 mon | Restrictive | Home-based intervention implemented primarily by participants' mothers | Children in the experimental group showed more improvement on measures of autism severity and social interaction, despite some improvement in the children in the comparison group |
| Brian, Smith, Zwaigenbaum& Bryson (2017) [29] | 62 children aged 16–30 mon | Restrictive | Social ABC's, parent intervention | Children in the experimental group showed significant gains over the control group in the following areas assessed: child functional vocal responsiveness to parent prompts, child vocal initiations, parent smiling, fidelity of implementation, and parent-reported self-efficacy |
| Oosterling et al. (2010) [43] | 75 children (52 boys) aged 12–24 mon | Loosely-defined | Parent intervention targeting joint attention | Additional non-intensive parental training did not have any influence on language and global clinical improvement outcome variables |
| Wetherby et al. (2014) [44] | 82 children aged 16–20 mon | Loosely-defined | Parent-implemented social intervention | Individual intervention improved social communication, daily living, receptive language, and social skills, while group intervention participants demonstrated worsening or no significant changes |
| **Uncategorized Behavioral Interventions**<br>studies that do not fit with any of the other categories | | | | |
| Landa, Holman, O'Neill, & Stuart (2010) [50] | 48 children (40 boys) aged 21–23 mon | Not defined | Interpersonal Synchrony or Non-Interpersonal Synchrony | Significant between-group differences for socially engaged imitation, but no significant between-group differences for shared positive affect, expressive language, or nonverbal cognition |
| Ingersoll (2010) [51] | 21 children (18 boys) aged 27–47 mon | Not defined | Reciprocal Imitation Training | Significantly more gains in elicited and spontaneous imitation for both objects and gestures, as compared to the control |

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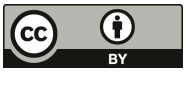

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brain sciences

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*Brief Report*

# Increased Neural Reward Responsivity in Adolescents with ASD after Social Skills Intervention

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Received: 22 May 2020; Accepted: 22 June 2020; Published: 24 June 2020

**Abstract:** The reward system has been implicated as a potential neural mechanism underlying social-communication deficits in individuals with autism spectrum disorder (ASD). However, it remains unclear whether the neural reward system in ASD is sensitive to behavioral interventions. The current study measured the reward positivity (RewP) in response to social and nonsocial stimuli in seven adolescents with ASD before and after participation in the Program for the Education and Enrichment of Relational Skills (PEERS®) intervention. This study also included seven neurotypical adolescents who were tested at two time points but did not receive intervention. We examined the RewP across the course of a task by comparing brain activity during the first versus second half of trials to understand patterns of responsivity over time. Improvements in social skills and decreased social-communication impairments for teens with ASD were observed after PEERS®. Event-related potential (ERP) results suggested increased reward sensitivity during the first half of trials in the ASD group after intervention. Adolescents with ASD who exhibited less reward-related brain activity before intervention demonstrated the greatest behavioral benefits from the intervention. These findings have implications for how neuroscience can be used as an objective outcome measure before and after intervention in ASD.

**Keywords:** autism spectrum disorder; EEG; ERP; reward response; RewP; sensitization; social skills intervention; PEERS®

## 1. Increased Neural Reward Responsivity in Adolescents with ASD after Social Skills Intervention

The cognitive process of habituation can be conceptualized in a variety of ways, but is generally considered a decreased response to stimuli after repeated exposure [1]. Individuals with autism spectrum disorder (ASD), defined by social communication deficits and the presence of restricted interests and repetitive behaviors [2], display altered rates of habituation. Specifically, individuals with ASD do not habituate to social information at the same rate as neurotypical controls, as evidenced through amygdala activation to faces over time [3–7]. In individuals with ASD, repeated presentation of social information elicits activation rates similar to that of novel stimuli for neurotypical subjects [8]. In neurotypical individuals, habituation tends to occur at a lower rate for stimuli that are more salient, intense, or stimulating [1,9]. Salient information may cause sensitization to stimuli, such that heightened responses can be observed over time [1,10]. One explanation for slowed habituation rates in response to faces is that individuals with ASD find processing social information more challenging than their neurotypical peers and thus must employ more cognitive resources. Alternatively, lack of habituation could reflect sensitization in this population.

Beyond reflecting the allocation of cognitive resources, habituation is also an indicator of learning. Reinforcement learning is facilitated by the goal of maximizing rewards and satisfying desired

outcomes. The reward system has been discussed at length in relation to the core symptoms of ASD. According to the social motivation hypothesis, individuals with ASD experience social interactions as less rewarding than their neurotypical peers, which may lead to reduced social initiation during critical periods of social development [11]. Investigations utilizing electroencephalography (EEG) to measure reward-specific event-related potentials (ERPs) suggest that children with ASD tend to find nonsocial stimuli more salient than social stimuli, and that children with ASD have less reward-related brain activity than that of their neurotypical peers in response to faces [12]. Thus, it is not that the reward system in ASD populations is under-active in response to all stimulus types, but that it is selectively functioning for some categories and not others [13]. However, the literature is mixed on whether the reward system is globally hypoactive in individuals with ASD [14,15]. If the reward system is selectively functioning in ASD, this system might be malleable, and behavioral intervention strategies that focus on social reinforcement might increase brain activity in response to social stimuli in this population. This hypothesis is supported by previous literature demonstrating neural changes in participants with ASD from pre- to post-intervention [16–22].

Social skills interventions for individuals with ASD often implement strategies of reinforcement learning, including applied behavior analysis and social skills training [23–25]. The goal of many interventions is to provide training for independent skill acquisition, ranging from a reduction in maladaptive behavior to increasing social engagement at school. Considerations of habituation or sensitization before and after such interventions are pertinent to not only the effectiveness of intervention but also the interpretation of outcomes.

Understanding how reward-related brain activity changes across the course of a task for individuals with and without ASD can increase our understanding of whether habituation or sensitization occurs at a similar rate across populations, and whether such activity is affected by participation in a social skills intervention. One method for measuring change in brain activity across a task is analyzing brain activity during the first and second halves of a task separately. In the current study, we sought to understand processes of habituation and sensitization to social stimuli among adolescents with ASD by examining patterns of reward-related neural responses to social versus nonsocial stimuli across a task (e.g., activity in the first versus second half of a task), before and after participation in a social skills intervention. The ERP task utilized was a reward-based guessing game in which participants were presented with rewards accompanied by incidental face or nonface stimuli.

## 2. Methods

### 2.1. Participants

Participants included seven adolescents with ASD, and seven age- and gender-matched neurotypical (TD) adolescents. Detailed information about participant demographics can be found in Table 1. No significant differences in age or IQ were observed between groups ($p$'s > 0.70).

For both the ASD and TD groups, exclusionary criteria included a history of seizures/epilepsy, a history of brain injury or disease, or a diagnosis of intellectual disability. For the TD group, immediate family history of ASD or developmental disabilities, or any psychiatric diagnosis for the adolescent was exclusionary. For the ASD group, a diagnosis of ASD was required, though commonly co-occurring disorders were not exclusionary (e.g., ADHD). For the ASD group, history of serious psychiatric illness (e.g., schizophrenia, bipolar disorders) or a recent (within 6 months) psychiatric hospitalization were exclusionary.

The study took place in inland Southern California with a large Latinx population [26]. Participant families were recruited via flyers posted online and via local community organizations. Those who expressed interest were contacted for an initial phone screen. At the initial intake appointment, informed consent and assent (from adolescents) were obtained.

**Table 1.** Descriptive characteristics of the autism spectrum disorder (ASD) and neurotypical (TD) groups.

| Variable | ASD | TD |
|---|---|---|
| Gender | 6 male, 1 female | 6 male, 1 female |
| Age in years, *M (SD), Range* | 13.88 (2.21), 11.26–16.98 | 13.46 (2.29), 10.10–17.10 |
| IQ, *M (SD), Range* | 104.14 (17.36), 77–129 | 102.50 (17.96), 79–128 |
| White *n* | 2 | 1 |
| Latino *n* | 4 | 4 |
| Mixed Race *n* | 1 | 2 |
| Maternal Education Level | | |
| Less Than College | 5 | 2 |
| College and Above | 2 | 3 |
| *Missing Data* | 0 | 2 |
| Household Income | | |
| Up to $50,000 | 3 | 1 |
| $50,001–$100,000 | 2 | 1 |
| Over $100,001 | 2 | 2 |
| *Missing Data* | 0 | 3 |

### *2.2. Behavioral Intervention (Program for the Education and Enrichment of Relational Skills, (PEERS®))*

PEERS® [25,27–29] is a manualized intervention designed to help adolescents make and keep friends (see [30] for intervention details). PEERS® consists of 16 weekly 1.5 h group sessions with concurrent but separate adolescent and parent groups. Parents learn how to support their adolescents in practicing and maintaining skills outside of the group. All groups were run by PEERS® certified providers.

## 3. Measures

Cognitive abilities were assessed using the 2-subtest Wechsler Abbreviated Scales of Intelligence [31] (WASI-II); an IQ under 70 was exclusionary for both groups. For adolescents with ASD, diagnosis was confirmed using the Autism Diagnostic Observation Schedule, Second Edition [32] (ADOS-2), and motivation to learn how to make and keep friends was assessed using the Mental Status Checklist [25]. Trained study staff performed these assessments. As these measures were used to confirm eligibility, they were only completed prior to the intervention.

### *3.1. Questionnaires*

Data reported here are part of a larger-scale study. Caregivers completed the Social Responsiveness Scale, Second Edition [33] (SRS-2) and the Social Skills Improvement System [34] (SSIS) both before the intervention began (Time 1), and immediately after intervention completion (Time 2). Times 1 and 2 were approximately 4 months apart. Neurotypical adolescents (TD participants) did not receive PEERS®, but had lab visits at Times 1 and 2, where each visit was four months apart. In addition, all adolescents completed the Test of Adolescent Social Skills Knowledge, Revised [27] (TASSK-R) at both Time 1 and Time 2, which measures acquisition of the concepts taught in PEERS®.

### *3.2. Electrophysiology Stimuli and Task*

The stimuli and task are described in detail in previously published manuscripts [12,35,36]. Briefly, the task was a guessing game in which participants saw a left and right visual stimulus (question marks), and were asked to indicate their guess via button press whether the left or right stimulus was "correct." After this choice, the left and right question marks were replaced with an arrow in the middle pointing towards whichever question mark the participant chose. This was done to reinforce the idea that participants had control over the task and their responses were being recorded.

In previously published manuscripts utilizing this task, participants were told that the reward for each correct answer was a small snack; here, the food reward was an Oreo cookie, or if preferred, fruit snacks or goldfish crackers. Participants were told that if they guessed correctly, they would see a ring

of intact Oreo cookies, and the cookies would be crossed out for incorrect answers. There were two blocked feedback conditions: Social versus nonsocial. Importantly, in both the social and nonsocial feedback trials, the face/arrow information was incidental (e.g., the face/arrow image was not part of the overt task). Thus, differences in brain activity between social and nonsocial conditions were not due to differences in tangible rewards or differences in task structure. Incidental stimuli in the social condition were faces obtained from the NimStim database [37] that were smiling for "correct" answers and frowning for "incorrect" answers. Incidental stimuli in the nonsocial condition were composed of scrambled face elements from the social condition formed into an arrow that pointed upwards for "correct" answers and downwards for "incorrect" answers. The order of social versus nonsocial blocks was counterbalanced between participants.

A computer program predetermined correct versus incorrect answers in a pseudorandom order, such that children got 50% "correct" and 50% "incorrect," with no more than three of the same answer-type in a row. The two feedback conditions (face/"social" trials and arrow/"nonsocial" trials) were tested in separate blocks, each composed of 50 trials.

### *3.3. EEG Recording*

Participants wore a standard, fitted cap (Brain Products ActiCap) with 32 silver/silver-chloride (Ag/AgCl) electrodes placed in accordance with the extended international 10–20 system. Continuous EEG was recorded using a Brain Vision Recorder with a reference electrode at Cz, and re-referenced offline to the average activity at left and right mastoids. Electrode resistance was kept under 50 kOhms. Continuous EEG was amplified with a directly coupled high pass filter (DC), and notch filter (60 Hz). The signal was digitized at a rate of 500 samples per second. Eye movement artifacts and blinks were monitored via horizontal electrooculogram (EOG) placed at the outer canthi of each eye and vertical EOG placed above and below the left eye. Trials were time locked to the onset of the feedback stimulus. To measure reward processing, the baseline period was −100–0 ms, and the data were epoched from −100 to 800 ms. Trials with no behavioral response, or containing electrophysiological artifacts, were excluded.

Artifacts were removed via a four-step process. Data were visually inspected for drift exceeding ±200 mV in all electrodes, high frequency noise visible in all electrodes larger than 100 mV, and flatlined data. Following inspection, data were epoched and eyeblink artifacts were identified using independent component analysis (ICA). Individual components were inspected alongside epoched data, and blink components were removed. To remove additional artifacts, we utilized a moving window peak-to-peak procedure in ERPlab [38], with a 200 ms moving window, a 100 ms window step, and a 150 mV voltage threshold.

For both conditions (face, arrow) and both feedback types (correct, incorrect), mean brain activity was calculated between 275 and 425 ms after feedback onset. The reward positivity (RewP) was defined as a difference wave, wherein brain activity in response to "incorrect" feedback was subtracted from brain activity in response to "correct" feedback. For statistical analysis, mean amplitude of the RewP between 275 and 425 ms was utilized. To compare reward-related brain activity during the first half and second half of trials, the first half and last half of all accepted trials (e.g., trials that were not removed through any of the processes mentioned above) were extracted for each of the two conditions (e.g., faces, arrows). Comparing brain activity during the first and second halves of trials allowed us to better understand patterns of reward-related brain activity throughout the task. To be included in statistical analysis, participants had to have a minimum of 6 trials in each half of each condition.

## 4. Results

All analyses were conducted using SPSS (version 26, Armonk, NY, USA). Prior to analysis, Pearson correlations between ERP amplitude, age, and IQ were conducted. No significant relationships were observed ($p$'s > 0.421).

### *4.1. ERP Results*

An independent samples t-test was conducted to ensure no significant differences in the number of acceptable trials were present between groups (all $p$'s > 0.638).

A 2 (group) × 2 (condition) × 2 (time) × 2 (half) repeated measure analysis of variance (ANOVA) was run. Condition (social, nonsocial), time (pre-intervention, Time 1; post-intervention, Time 2), and half (RewP amplitude during the first and second halves of the task) were within-subjects variables, and group (TD, ASD) was used as a between-subjects variable. A significant 3-way interaction was found between time, half, and group; $F(12, 20.76) = 5.20$, $p = 0.042$, $\eta_p^2 = 0.30$. Pairwise comparisons revealed a significant effect of group, such that the ASD group had significantly larger RewP amplitude compared to that of the TD group in the first half of trials at Time 2; $F(12, 27.04) = 4.83$, $p = 0.048$. Thus, regardless of condition, the ASD group had larger reward-related brain activity in the first half of presented trials at Time 2 (post-intervention) compared to that of the TD group. No other significant main effects or interactions were observed. See Figure 1 for grand average waveforms at Time 2.

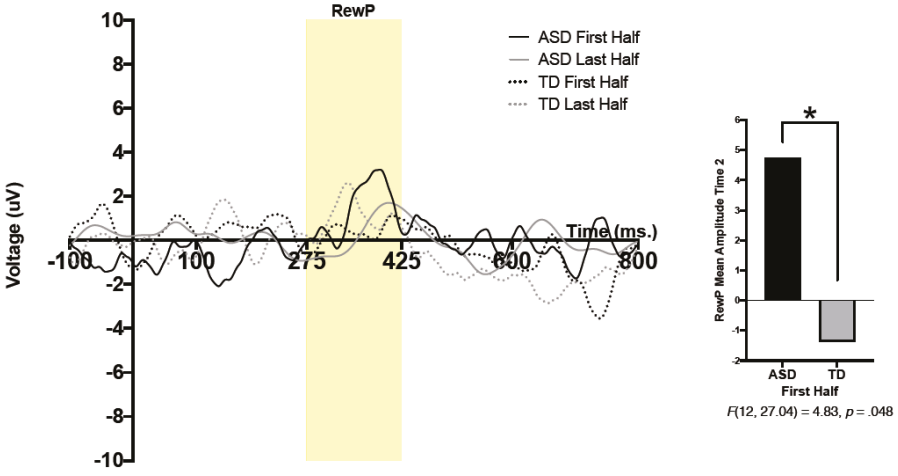


**Figure 1.** Grand average waveforms during the first and second halves of trials in participants with and without ASD at Time 2 (post-intervention). Significant differences were observed between the ASD and TD groups during the first half of trials at Time 2 (post-intervention). Note that for the purposes of this figure, the ERP was filtered using a 25 Hz low-pass filter. * $p < 0.05$.

### *4.2. Behavioral Results*

To understand how behavioral measures changed over time for each group, 2 (group) × 2 (time) repeated measure ANOVAs were conducted on measures of autism symptoms (SRS-2), social skills (SSIS social skills subscale), and PEERS®-specific knowledge (TASSK-R).

For the SRS-2, a main effect of group was observed, $F(1,12) = 9.51$, $p = 0.009$, $\eta_p^2 = 0.96$, such that the TD group had significantly lower SRS-2 scores than those of the ASD group. Lower SRS-2 scores indicate less severe social impairments. An interaction between group and time approached significance, $F(1, 12) = 4.56$, $p = 0.054$. Post-hoc follow-up tests using Bonferroni corrections revealed a significant difference between groups on the SRS-2 at Time 1 (pre-intervention), such that the TD group had lower scores than those of the ASD group ($p = 0.001$). The difference between the two groups was no longer significant at Time 2 (post-intervention). Pairwise comparisons revealed a trend-level effect of time for the ASD group, such that SRS-2 scores decreased from pre- to post- intervention ($p = 0.07$), whereas no effect of time was observed for the TD group.

For the SSIS social skills subscale, an interaction between group and time approached significance, $F(1,12) = 4.20$, $p = 0.063$. Post-hoc follow-up tests using Bonferroni corrections revealed a significant

effect of time for the ASD group, such that SSIS social skills subscale scores increased from pre- to post-intervention ($p = 0.035$), whereas no effect of time was observed for the TD group. Higher scores on the SSIS social skills subscale indicate better social skills. Pairwise comparisons also revealed a trend-level difference between groups on the SSIS social skills subscale at Time 1 (pre-intervention) such that the TD group had higher scores than those of the ASD group ($p = 0.071$), whereas the difference between groups was not significant at Time 2 (post-intervention).

For the TASSK-R, a main effect of group was observed, $F(1,12) = 5.4$, $p = 0.038$, $\eta_p^2 = 0.31$, such that adolescents with ASD had higher scores on the TASSK-R compared to neurotypical teens. Higher scores on the TASSK-R indicate more understanding of PEERS®-specific skills. A significant effect of time was observed, $F(1,12) = 45.82$, $p < 0.001$ $\eta_p^2 = 0.79$, such that TASSK-R scores increased from Time 1 (pre-intervention) to Time 2 (post-intervention). A significant interaction between time and group was observed, $F(1,12) = 25.78$, $p < 0.001$, $\eta_p^2 = 0.68$. Post-hoc follow-up tests using Bonferroni corrections revealed a significant effect of time for the ASD group, such that scores on the TASSK-R increased from pre- to post-intervention ($p < 0.001$). No effect of time was observed for the TD group. Pairwise comparisons also revealed a significant difference between groups on the TASSK-R at Time 2 (post-intervention), such that the ASD group had higher scores on the TASSK-R compared to those of the TD group ($p = 0.001$), whereas the difference between groups was not significant at Time 1 (pre-intervention). Please refer to Table 2 for behavioral measures at each timepoint.

**Table 2.** Behavioral measures for Time 1 and Time 2 in ASD and TD groups.

| Variable | ASD | TD |
|---|---|---|
| Time 1 *M (SD), Range* | | |
| SRS-2 | 69.14 (14.18), 47–90 | 44.00 (4.55), 39–52 |
| SSIS Social Skills | 85.86 (25.13), 41–121 | 106.71 (11.93), 94–125 |
| TASSK-R | 14.29 (3.09), 10–9 | 14.57 (3.69), 10–21 |
| Time 2 *M (SD), Range* | | |
| SRS-2 | 61.43 (14.89), 45–88 | 48.00 (14.46), 39–80 |
| SSIS Social Skills | 93.57 (22.78), 51–120 | 105.00 (9.27), 96–119 |
| TASSK-R | 24.29 (4.61), 17–29 | 16.00 (2.65), 14–21 |

### *4.3. Brain and Behavior Correlations*

Within the ASD group, Pearson correlations were conducted to examine how change on the behavioral measures from pre- to post-intervention related to ERP results. Difference scores were calculated for the SRS-2, SSIS social skills subscale, and TASSK-R by subtracting post-intervention scores from pre-intervention scores. A significant negative correlation was observed between the SRS-2 difference score and RewP amplitude in the last half of the social condition at Time 1 ($r = -0.77$, $p = 0.044$), such that participants with ASD who had less reward-related brain activity in response to social stimuli at Time 1 (pre-intervention) displayed larger improvements on the SRS-2 compared to individuals with more robust social reward-related brain activity at Time 1. See Figure 2A.

A positive correlation was observed between RewP amplitude in the last half of the social condition at Time 1 (pre-intervention) and SSIS social skills subscale difference score ($r = 0.78$, $p = 0.038$), such that adolescents with ASD who displayed less social reward-related brain activity during the last half of trials in the social condition at Time 1 exhibited greater improvements in social skills from pre- to post-intervention compared to those who displayed more robust reward-related brain activity prior to intervention. See Figure 2B.

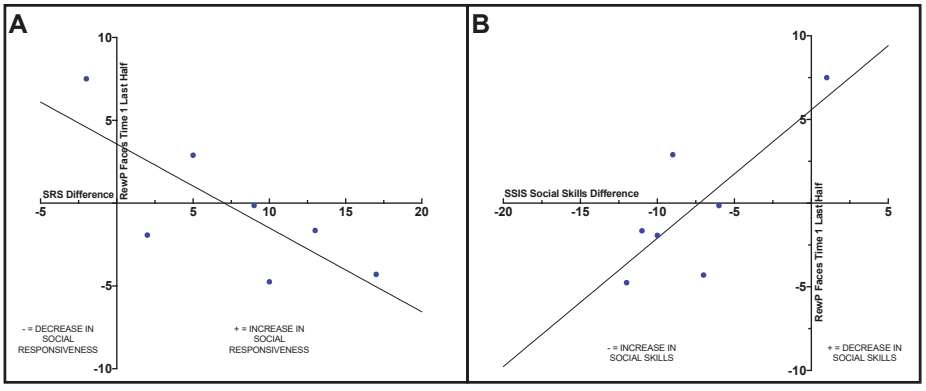


**Figure 2.** (**A**) Correlation between SRS-2 difference score before and after intervention in the ASD group and reward positivity (RewP) mean amplitude in the last half of the social condition at Time 1 ($r = -0.77$, $p = 0.04$). (**B**) Correlation between SSIS social skills difference score before and after intervention in the ASD group and RewP mean amplitude in the last half of the social condition at Time 1 ($r = 0.78$, $p = 0.04$).

Finally, a negative correlation was found between the TASSK-R difference score and RewP amplitude in the last half of the social condition at Time 2 (post intervention) ($r = -0.79$, $p = 0.035$), such that participants with ASD who demonstrated larger increases in their knowledge of intervention-specific knowledge displayed larger social reward-related brain activity in response during the second half of trials compared to participants who had smaller increases in intervention-specific knowledge from pre- to post-intervention.

No significant correlations were observed between behavioral measures and reward-related brain activity in the nonsocial (arrow) condition.

## 5. Discussion

This study investigated the effect of the PEERS® social skills intervention on both neural correlates of reward processing and social behaviors in adolescents with ASD. Specifically, we sought to understand how reward-related brain activity changed throughout the course of a task by comparing brain activity during the first and second halves of trials.

Prior to the start of the intervention, patterns of reward-related brain activity did not differ between participants with ASD and their neurotypical peers. However, after intervention, participants with ASD were more sensitive or responsive to all reward types (both social and nonsocial) during the first half of the ERP paradigm. Increased brain activity related to reward processing indicated increased reward responsivity in adolescents with ASD, irrespective of stimulus type, after participating in a social skills intervention. A larger reward response is similar to what Kohls and colleagues [14] have described as a "liking" response involving the consumption of rewards that are salient. Initial sensitivity to rewards (e.g., during the first half of trials) may have been heightened after exposure to frequent reinforcement strategies that were utilized throughout the intervention to encourage participant engagement.

Although lack of significant differences in brain activity between groups at Time 1 (pre-intervention) is in contrast with some previous intervention literature utilizing neuroscience methods, e.g., [16], and changes in brain activity from pre- to post- intervention in individuals with ASD has been reported previously [17,18,20,21]. Notably, previous research measuring brain activity before and after intervention in individuals with ASD either did not utilize a neurotypical control group, e.g., [17,18,20,21], or had a neurotypical group but did not test children with ASD and the TD group at two timepoints (e.g., pre- and post-intervention for the ASD group). [16,22]. Collecting data from both teens with ASD and their neurotypical peers, as well as utilizing neuroscience paradigms that

are hypothesized to capture changes directly relevant to the intervention itself, are both important strategies when measuring neural correlates of change after an intervention (for a review, see [39]). In the current study, we hypothesized that increased reward-related brain activity would be observed across the course of the ERP task after teens with ASD underwent an intervention that utilized social positive reinforcement principles to increase success in making and keeping friends. To our knowledge, this is the first investigation of brain activity of both neurotypical teens and those with ASD before and after participation in an intervention (or, in the case of the TD group, before and after a delay in which no intervention took place).

Contrary to our hypotheses, brain activity did not differ in response to condition (e.g., social, nonsocial) for either group. This contrasts with previous findings using this paradigm with young children with and without ASD [12,35]. However, this is the first time that this ERP paradigm has been utilized with adolescents. Thus, differences between the current study and previous research might reflect developmental changes. It is plausible that adolescents with and without ASD are less overtly motivated by food rewards as they would be by other reward types (e.g., monetary), and thus may have found the paradigm less engaging/rewarding than younger children. Future studies should consider utilizing this paradigm in a cross-sectional design with different age groups to better understand the effects of age on reward responsivity.

As expected, at Time 1 (pre-intervention), the ASD group had more severe social-communication impairments associated with ASD (measured by the SRS-2) and poorer social skills (measured by the SSIS social skills subscale) than the TD group. Adolescents with ASD improved on both measures after intervention (Time 2), which mirrors previously reported findings of the effectiveness of the PEERS® social skills intervention [29,30]. No differences were observed from Time 1 to Time 2 in the TD group. This was expected, as the neurotypical teens did not participate in the intervention. Importantly, only one ASD participant remained in the range for clinical concern on both the overall SRS-2 score and SSIS social skills subscale score following intervention. This is important as it suggests that change from Time 1 to Time 2 was not only statistically significant, but also clinically meaningful. Further, no significant differences were observed between groups on the SRS-2 or SSIS social skills subscale at Time 2 (post-intervention), suggesting that both social-responsiveness symptoms and social skills in our sample of adolescents with ASD began to resemble social behaviors observed in our neurotypical participants.

One of the most interesting findings of our investigation was that ASD participants who demonstrated less robust social reward-related brain activity in the second half of trials prior to the intervention (Time 1) evidenced the biggest gains from Time 1 to Time 2 in both social responsivity and social skills. This suggests that perhaps the adolescents who benefitted the most from PEERS® were those who had the most "room to improve" in terms of social reward response. This also provides initial evidence that the neural characteristics of reward responsiveness prior to intervention may serve as an indicator of treatment response. That is, it might be possible to utilize neural correlates of social reward responsivity to predict which individuals with ASD might benefit the most from participating in PEERS®. To further investigate this potential predictor of intervention efficacy, future research with a larger sample size and a randomized control group should be conducted.

## 6. Limitations

This study is part of a larger investigation of a social skills intervention, and this report serves as an initial analysis. Thus, the current study had a small number of participants. It is important to interpret differences in behavioral measures that were approaching significance with caution. Additionally, randomization of treatment was not performed (i.e., a waitlist control group was not utilized) and ASD participants were aware of their enrollment in the social skills intervention (i.e., parent rating forms were not completed "blind," as parents were actively participating in the PEERS® intervention with their teen). Thus, we cannot rule out the possibility that improvements in parent ratings in the ASD group were due to the expectation of improvements. Finally, findings from this study

cannot be generalized to all individuals with ASD, as one of the criteria for participation was that the adolescent was motivated to participate in PEERS® and wanted help making and keeping friends. Thus, this sample consisted of adolescents who were highly motivated to learn social skills.

## 7. Conclusions

The results of our study have important implications for intervention outcomes in adolescents with ASD. First, these findings add to the existing literature on the efficacy of PEERS® for adolescents with ASD. Second, we found evidence for increased reward sensitivity in adolescents with ASD (compared to their neurotypical peers) after participation in the intervention. This suggests that participating in PEERS® increases reward system sensitivity in teens with ASD. Finally, we found that teens who benefitted the most from the intervention (i.e., had the largest gains in social skills and largest decrease in social-communicative impairments) were those with less reward-related brain activity in response to faces prior to the intervention. This relationship between symptom improvement and brain activity prior to the intervention suggests that PEERS® might be most effective for teens with ASD who have "room to grow" in their social reward responsivity, whereas teens with ASD who already have higher levels of social reward responsivity might benefit less. Finally, neuroscience measures may be reliable predictors of teens' responsiveness to treatment because they are independent of potentially biased parent ratings.

**Author Contributions:** K.K.M.S. designed the experiment. E.B. and K.K.M.S. conceptualized the analysis strategy. E.B. performed the EEG processing and statistical analysis under the supervision of K.K.M.S. J.B. verified the analytical methods and interpretations. E.V. and A.M.M. reviewed and confirmed descriptions of methodology. All authors discussed the results and contributed to the final published manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the Bezos Family Foundation Grant "Autism, the 'Social Brain,' and Neuroscience: Treating Underserved Latino Teenagers in the Inland Empire."

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Case Report*

# Improving the Ability to Write Persuasive Texts in a Boy with Autism Spectrum Disorder: Outcomes of an Intervention

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Received: 12 March 2020; Accepted: 28 April 2020; Published: 30 April 2020

**Abstract:** In this paper, we describe an intervention implemented to assist a 13.2-year-old boy with Autism Spectrum Disorder, G, without intellectual disability, aimed at improving his ability to compose persuasive texts. There was an initial assessment (baseline), an intermediate assessment after two weeks, a six-session intervention phase, and a post-intervention assessment. Our intervention applied two procedures. The first aimed at enhancing general composition abilities in terms of picking (P) ideas, organizing (O) notes, and writing (W) them down (POW), while the second specified the steps to write a persuasive text addressing a possible reader: a topic sentence (T), reasons (R), an explanation (E) for the reasons and the end of the sentence (E) (TREE). These procedures were termed POW + TREE. To analyze G's texts, three types of measures were used by two raters at baseline, intermediate and post-test time: (a) the presence of the TREE components; (b) the quality of the reasons and explanations for the reasons; (c) the number of mental state terms. All these measures showed relevant quantitative improvements, as well as qualitative changes. In addition, when G's performance at the end of the intervention was compared to that of typically developing controls, no statistical difference appeared. The results are discussed in light of the potentialities offered by the type of intervention described here.

**Keywords:** persuasive text writing; perspective-taking; autism spectrum disorder; adolescence; intervention

## 1. Introduction

Autism Spectrum Disorder (ASD, henceforth) is an umbrella expression that designates a set of heterogeneous early onset neurodevelopmental conditions. In general terms, these conditions are characterized by well-known patterns of specific behaviors during social interaction and communication, and unusually restricted and repetitive activities and interests [1]. Prognostic studies suggest better outcomes in individuals with ASD who possess a higher intellectual level, relatively fluent language at the beginning of primary school, and reduced difficulties in social abilities. Actually, follow-up studies show a plurality of developmental trajectories in children with ASD (for a review, see Lai, Lombardo and Baron-Cohen [2]).

In the learning area, this clinical population, even without intellectual disability, generally shows heterogeneous profiles. For instance, in a study conducted on 100 adolescents (mean age: 15.6) by Jones and colleagues [3], in terms of reading and mathematics competence, the authors found "peaks and dips" in the profiles of the participants. In every participant, there was at least one ability that was markedly over or under the expected level. In a similar vein, Randi, Newman and Grigorenko [4], who reviewed studies on the profiles of readers with ASD, found that these profiles were extremely variable.

In the area of writing, children with ASD shown similar heterogeneous skills (see Zajic and Wilson [5]). Some children with ASD show well-developed writing skills and may even become skillful writers [6], while others manifest difficulties that place them below their typically developing peers' levels [5,7]. In a recent meta-analysis, Finnegan and Accardo [7] identified six critical components in the writing abilities of individuals with ASD compared with their typically developing peers, namely handwriting length, legibility, size, speed, spelling and structure, while no difference appeared in sentence construction. The factors that might account for differences in these critical components are still under study. Nevertheless, according to Accardo and colleagues, and Zajic and Wilson [5,8], it is highly plausible that differences in the texts produced by individuals with ASD are related to Theory of Mind [2], executive function, fine motor skills and/or speech and language skills. Another source of variability could be associated to the type of texts these individuals are faced with. In particular, persuasive text seems to be one of the most difficult [9] due to the following reasons.

As the goal of a persuasive text is to persuade a reader about the value of some arguments, overcoming all possible counter-arguments, the writer's concern is to argue his/her opinions on a given topic, provide reasons to support these opinions, and defend them. To sum up: composing a persuasive text requires the writer to adopt the interlocutor's point of view and revert it by using even stronger arguments. To this end, the interlocutor's arguments must be taken into account but also overridden by further, undisputable arguments. All these operations make the writing of a persuasive text a particularly sophisticated communicative task.

In particular, a persuasive text requires competence in the very object of debate, turn-taking ability as a component of Theory of Mind, an ability to weigh the various facets of the issue at hand, an ability to construct an appropriate synthesis of both the arguments and the counter-arguments, which, in turn, requires integrative processing skills [2]. In addition, there are also linguistic requirements such as appropriate vocabulary, particularly concerning mental states (epistemic and emotional–volitional words and expressions), inter- and intra-propositional cohesion, and knowledge of typical rhetorical devices in writing. For instance, a persuasive text must contain connectives such as "that is" (to be precise), "indeed" (to present evidence), "therefore" (to draw conclusions). Lastly, the sequence of statements is guided essentially by logical, rather than temporal criteria, which entails the use of other types of connectives ("in addition", "as a consequence", "in summary", "overall", "in conclusion") [9–11].

Brown, Johnson, Smith and Oram Cardy's [11] study, based on two groups of adolescents, one of 25 students (mean age: 12), with ASD but without linguistic impairment, and another group of 22 typically developing students (mean age: 13), apparently supports the above hypothesis. The participants had to read a series of directions aimed at writing persuasive texts on a screen. The main differences between the two groups concerned all production measures (examples the number of words), lexical and syntactic complexity, quality of the arguments, but not cohesion measures and writing conventionalities. The authors interpreted the lower quality of the texts produced by the participants with ASD in light of Flower's concept [12] of "writer-based text", as opposed to "reader-based text". The former was thus termed because it does not take into account the reader's perspective and is characterized by two main features: insufficient integration between components into a higher-order framework, which results in lists of details instead of a general concept, and insufficient clarity, due to over-vague and ambiguous referencing.

In more recent years [8,9,13], there has been a growing interest in the multiple procedures, often used in combination, which adults can apply to support the writing process in individuals with ASD. Concerning persuasive text, certain types of interventions have specifically aimed at inducing a shift from a writer-based perspective to a reader-based perspective, as indicated by Brown and colleagues' study [11]. These authors suggested: (a) the use graphic organizers as tools to support the planning phase (pre-writing activities); (b) to teach how to graduate from factual details to higher-order concepts; (c) to teach participants how to weigh the strength of each individual argument as a basis for organizing the whole argument; (d) to provide participants with visual supports to recall the various steps of

the writing process; and (e) to encourage students to ask for feedback from readers [11]. All these suggestions are also mentioned in the research synthesis by Accardo and colleagues [8]. Asaro-Saddler and Saddler [14], and Asaro-Saddler and Bak [15], investigated the possibility of enhancing this type of writing using the Self-Regulated Strategy Development (SRSD) program, which was originally developed by Graham and Harris [16,17]. This program aims at teaching planning, stimulating a flexible use of strategies, and promoting both a positive attitude towards writing and a positive self-image as a writer. This study implemented two lessons and mnemonics that were also implemented in Asaro-Saddler and Bak [15]. The first aimed at enhancing general composition abilities in terms of picking (P) ideas, organizing (O) notes, and writing (W) them down (POW), while the second specified the steps to write a persuasive text addressing a possible reader: a topic sentence (T), reasons (R), an explanation (E) for the reasons and the end of the sentence (E) (TREE). The participants were three children with ASD, between eight and nine years old. The authors compared three persuasive baseline essays with three post-intervention texts and found evident improvements, both in qualitative and quantitative aspects, which gives support to the effectiveness of the POW + TREE approach. Asaro-Saddler [18], after reviewing 11 studies investigating the specific strategy of SRSD used in the writing instruction of learners with ASD, found that these students improved their planning ability, the number of written elements, and the content of their writing when using the self-regulated strategy.

In our study, we applied a program with a boy, conventionally called "G", with ASD and without intellectual disability, but with clear difficulties in writing persuasive texts, as attested by his teachers. In this article, we will consider G's change over six persuasive writing tasks: two at baseline, two after two weeks, and two after the intervention. In addition, we also considered the performance of a control group of typically developing children in the last two persuasive writing tasks, written at the same point in the school year, and compared G's performance to that of the controls. While an increase in G's overall performance was expected as a function of the intervention, it was more difficult to foresee whether there would be differences, and in which areas these differences might be found, between G's performance and that of the controls who had not undergone any intervention at all regarding persuasive text composition.

## 2. Materials and Methods

To evaluate the outcomes of the intervention implemented with G, we analyzed his change in text composition, starting from an initial assessment (baseline), followed by an intermediate assessment after two weeks, itself followed by a six-session intervention, and lastly by a post-intervention assessment. We also compared the last phase of G's production to the persuasive text composition of typically developing children ($n = 8$) enrolled in the same school grade (mean age: 13.5 at G's post-test time). The study was approved by the Ethics Committee of the Department of Developmental and Social Psychology, "Sapienza" University of Rome. Informed consent was given freely by G's and the controls' parents.

### 2.1. Participants

G was a 13.2-year-old boy at the beginning of the intervention, and was enrolled in grade 8 in an Italian public school. He had been diagnosed as a child with ASD without intellectual disability. A first diagnosis, based on DSM-IV-TR [19], was then confirmed on the basis of the DSM 5 criteria [1] at the age of 11. The instruments used were the Autism Diagnostic Observation Schedule—Second Edition (ADOS—2) [20], an interview with G's parents and teachers, focused on the social and communicative aspects of G's behavior, and his learning profile.

In the social area, there was a clear discrepancy between G's interaction abilities with adults or with peers, with the former being more adequate. Among the reasons that made G's interaction with his peers problematic, we must mention his difficulty in perspective-taking, his erudite language, and over-developed moral rigidity. G also developed specific knowledge on some topics, most often felt by his interlocutors as too sophisticated and cultivated. For instance, faced with a given conflict between

his friends, G responded with a political reference, mentioning the Foreign Affairs Ministry and the Internal Affairs Ministry, using the corresponding metonymical expressions ("Farnesina" (the British equivalent of "Farnesina" would be "Downing Street") for the former, and "Viminale" for the latter). Lastly, in everyday routines, G would show some dysfunctional behavioral patterns that appeared difficult to modify.

As mentioned above, G's intellectual level, as measured by the Intellectual Quotient of the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV) [21], was average (IQ: 107) although it was not representative of his performance across each index: Verbal Comprehension Index (126); Perceptual Reasoning Index (124), Working Memory Index (85), Processing Speed Index (71). In contrast, G's General Ability Index (128), which is based on the first two indices, can be considered as representative. It must be noted that the weighted score for Vocabulary (18), based on word definition, places G in the very above average range. His grammatical comprehension score (108), as measured by the Test for Reception of Grammar, 2nd edition (TROG 2) [22], was also average. His sentence production abilities were very good (z: 1.45), as measured by Gugliotta and colleagues' test [23], as well as his verbal reasoning (z: 1.17), measured by the same test, which assesses the capability to identify absurd statements in sentences, understand proverbs, identify a super-ordinate category, and differences in word pairs.

In his academic abilities, G showed some strengths and weaknesses. For instance, in a standardized Italian reading test [24], his comprehension performance was adequate both for accuracy and speed, reaching the 90th percentile. In contrast, G's handwriting appeared slow, as measured by an Italian test [25] that evaluates writing speed, subdivided into three parts, each of which are performed in one minute: (a) writing the two graphemes "l" and "e" in italics in a continuous way; (b) writing the Italian word "uno" (Eng: "One") as many times as possible; (c) writing as many words designating numbers as possible. The z scores were as follows: (a) within norm; (b) z: −1.43; (c) z: −2.09. Despite the above praxic difficulties in writing, G's teachers reported that the child was perfectly able to compose descriptive and narrative texts using the font he had better automatized, namely capital script, while he was very poor at composing persuasive texts.

The controls were recruited randomly in the same classroom as G's in a school attended by families sharing the same sociocultural background, without learning disabilities nor any other type of developmental disorder. The whole classroom had followed a standard school curriculum, without a specific focus on argumentative text as in the program implemented with G. Based on teacher's quantitative assessment, the controls' performance in persuasive texts ranged from adequate to good, and for this reason we did not assess their competence in this type of text at baseline.

### *2.2. Intervention Procedures*

Our intervention focused on teaching two mnemonics implemented in earlier research studies with children with ASD [15]. The first aimed at enhancing general composition abilities in terms of picking (P) ideas, organizing (O) notes, and writing (W) them down (POW), while the second specified the steps to write a persuasive text addressing a possible reader: a topic sentence (T), reasons (R), an explanation (E) for the reasons and the end of the sentence (E) (TREE). As an extension of the explanation category, which represents the core of the argumentation process, we also considered counter arguments (C.Arg), i.e., those arguments that are in favor of the reader's perspective. We will therefore describe the activities implemented in each session not as an abstract schema, but as the actual sequence applied to G's case, namely: modeling (1), joint writing (2), guided writing (3), autonomous writing (4). Therefore, the POW + TREE procedures were applied flexibly as a function of G's reactions.

Each session lasted about 90 min. In the first session (modeling), the adult illustrated the aim of the session and interactively analyzed the meaning of the expression persuasive text (PT), defined as: "A PT tells the reader what the writer believes or thinks about a particular topic".

The adult explained and modeled POW and TREE using a thinking-aloud procedure. In the planning phase (P), the adult would make the following type of suggestion: "To write my text, the problem must be very clear in my mind . . . I must have some idea about the topic . . . and therefore, I might have to search for information in books, on the Internet, or ask the others, etc . . . ". In the organization phase (O), the adult would say: "To convince the reader that my opinion is a valid one, I must organize my thoughts in a logical manner: first, I will state the problem at hand and my personal opinion . . . then, I will give my reasons and try to explain them the best I can . . . and, in the end, I will draft a conclusion".

In the writing phase (W) the adult would say: "Now I am going to write the text based on the POW I wrote before". In this process, he would call into question his linguistic choices: "Will the reader understand my idea the way I phrased it?", and justify them: "Maybe here it's better to write "indeed", because I wish to prove my reasons", etc.

In this session, the adult composed a PT on how to persuade a frequent video game user not to stick to the screen at the expense of more constructive activities, such as social exchange with friends or reading interesting things. In order to model the second TREE component (R), the adult argued that video games can cause severe addiction (R1); that not every video game is as stimulating as other games in real life (R2); that excessive video-game playing can impoverish one's social life with peers (R3). Once the text was complete, the adult and G identified the TREE components and transferred them into the graphic organizer (Figure 1).

| **T—Topic sentence: what do I believe?** |
| --- |
| |
| **R—Reasons: 3 or more: Why do I believe this? Will my reader also believe this?** |
| |
| **E—Explain Reasons/or provide counter argument: I will explain each reason. But, on the other hand…** |
| |
| **E—Ending: now it's time to wrap up** |
| |

**Figure 1.** Topic sentence (T), reasons (R), explanation (E) and end of the sentence (E) (TREE) graphic organizer—Graham and Harris. Adapted from Asaro-Saddler and Bak [15] with some modifications.

At the beginning of the second session (joint writing—first step), the adult showed G the two texts he had written during the baseline phase and invited him to identify the TREE components. To facilitate the task, the adult asked G to fill the slots of the graphic organizer (Figure 1) and, in relation to each component, G wrote the action that best fit the meaning of it.

In the third session (joint writing—second step), the adult chose a text from a book and analyzed the TREE components jointly with G in order to check G's comprehension. Afterwards, the adult and G interactively wrote a further PT text. The adult supported G during the writing process, in particular when the child omitted something important, and also mitigated G's negative thoughts, such as: "I really have no idea", "I really don't see how to do that". To contrast the child's negative feelings, the adult used self-reinforcing sentences, such as: "I remember once I could overcome more difficult obstacles than this"; "In the future, what I am doing now might prove helpful". G had a list of these self-reinforcing sentences, named the "thought chart", which he could consult at any time.

In the fourth session (guided writing—first step), G had to fill up his TREE graphic organizer by himself. The adult did not intervene anymore regarding the text, but stimulated G to follow the various steps of the POW procedure, and encouraged him to use the thought chart. At the end of the composition, G was invited to check the presence of all the components in his text, as represented by the image of a rocket (Figure 2). In other words, G was told that the "rocket" could not start if the components were not all present. In the fourth session, G produced two PTs.

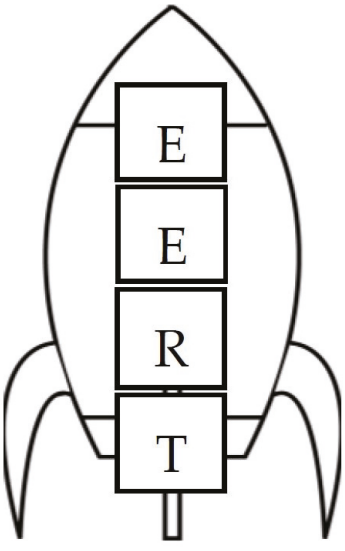


**Figure 2.** The rocket image to check the presence of the TREE components.

In the fifth session (guided writing—second step), G did not need to base his writing on the TREE graphic organizer anymore, because he had already memorized it, and rehearsed the POW procedure by himself. The adult just reminded G to use the thought chart. In this fifth session, G produced two PTs.

In the sixth and last session (autonomous writing), G wrote two PTs in a totally autonomous way, sometimes rehearsing the POW procedure by himself and recalling some of the self-reinforcing sentences of the thought chart.

### *2.3. Measures*

To analyze the outcomes of the intervention in terms of the level of the PTs produced (G was allowed to choose the font he had better automatized, which was capital script), a series of criteria were applied, partly inspired by Asaro-Saddler and Bak's study [15], blending quantitative and qualitative aspects. The topics of the PTs had been chosen based on the interests that motivated G, as reported by his parents, and those which could be shared with his peers: the use of mobile phones, McDonald's restaurants, holidays at the seaside, bad experiences with animals, reasons for not going to school during summer and the use of social networks. The following is an example of directions for composing a persuasive text: "Your friend had a bad experience: he was bitten by a dog. From that moment onwards, he did not want to have contact with any kind of animal anymore. Try to write a text to convince him to approach the animal world again".

Two independent raters, who did not know the nature of the intervention nor the sequence of the text composition, analyzed the six texts written by G and the two texts written by the controls on the basis of three criteria: (1) the presence/absence of the TREE components (topic, reasons, explanation/counter

argument, ending); (2) the qualitative level of the reasons and explanations/counter arguments; (3) the amount of mental state terms.

For the first criterion, the scores varied depending on the components. For topic and ending the scoring was dichotomous: the absence of a topic or ending was worth no points, and the presence of topic or ending was worth one point. For reasons and explanations/counter arguments, the score varied as a function of the number of reasons or explanations/counter arguments provided by the participants. For example, one reason or one explanation/counter argument was worth one point, two reasons or explanations/counter arguments were worth two points, etc.

As for the second criterion, the qualitative level of reasons and explanations/counter arguments, a four-point scale (zero to three) was applied, one for each criterion separately. For reasons, a score of zero was attributed to no reasoning or irrelevant reasoning; a score of two was attributed to ill-focused reasoning, a score of two was attributed to relevant but non exhaustive reasoning, and a score of three was attributed to exhaustive reasoning. For explanations/counter arguments, a score of zero was attributed to no explanation or irrelevant explanation/no counter argument or irrelevant counter argument; a score of one was attributed to ill-focused explanation/ill-focused counter argument; a score of two was attributed to relevant but non exhaustive explanation/relevant but non exhaustive counter argument, and a score of three was attributed to exhaustive explanation/exhaustive counter argument.

To assess the third criterion, i.e., the amount of mental state terms, the rater had to identify and count two categories of words or expressions: epistemic and emotional–volitional. The score resulted from the total number of these words or expressions in each text.

- Epistemic verbs: "I know/I don't know; I think/I don't think; I believe/I don't believe", etc.;
- Epistemic locutions: "it seems to me; to me", etc.;
- Epistemic nouns: an idea; a thought; an opinion, etc.;
- Emotional–volitional verbs: I like; I do not like; I want, etc.;
- Emotional–volitional nouns: pleasure; disgust, etc.;
- Emotional–volitional adjectives: marvelous; horrible, etc.

## 3. Results

Table 1 reports the scores of all the measures considered in G's PTs. We can observe that the scores related to the presence/absence of the TREE components increased from five to six from the initial to the intermediate phase and then markedly improved up to 20 in the post-test and, in particular, in the final PT, where G gave four reasons and four explanations. As for the scores assessing the qualitative aspects of reasons and explanations/counter arguments, there is an almost exponential improvement: 2–4–18 for the reason scale, and 0–5–20 for the explanations/counter arguments scale.

The scores assessing the separate amount of epistemic and emotional–volitional terms, and the total amount of mental state terms also show a very relevant improvement, where the total score increases from six, to 11, and then to 23, despite some discrepancies between epistemic and emotional–volitional terms in some areas.

**Table 1.** Scores of all the measures in G's persuasive texts (PTs) in all phases.

| | Baseline | | Intermediate | | Post-Test | |
|---|---|---|---|---|---|---|
| | **PT1** | **PT2** | **PT3** | **PT4** | **PT5** | **PT6** |
| Topic | 0 | 0 | 0 | 0 | 1 | 1 |
| Reasons | 2 | 1 | 2 | 1 | 3 | 4 |
| Exp/C.Arg. | 1 | 1 | 1 | 2 | 5 | 4 |
| Ending | 1 | 0 | 0 | 0 | 1 | 1 |
| Total PT | 3 | 2 | 3 | 3 | 10 | 10 |
| Total Phase | 5 | | 6 | | 20 | |
| Reason 1 | 0 | 0 | 2 | 2 | 3 | 2 |
| Reason 2 | 2 | - | - | - | 3 | 2 |
| Reason 3 | - | - | - | - | 3 | 2 |
| Reason 4 | - | - | - | - | - | 3 |
| Exp/C.Arg 1 | - | 0 | 1 | 2 | 3 | 3 |
| Exp/C.Arg 2 | - | - | - | 2 | 3 | 3 |
| Exp/C.Arg 3 | - | - | - | - | 2 | 3 |
| Exp/C.Arg 4 | - | - | - | - | 0 | 3 |
| Exp/C.Arg 5 | - | - | - | - | 0 | - |
| Total Reasons | 2 | | 4 | | 18 | |
| Total Exp/C.Arg | 0 | | 5 | | 20 | |
| Epist | 3 | 0 | 9 | 1 | 6 | 9 |
| Em–Vol | 0 | 3 | 1 | 0 | 4 | 4 |
| Tot Epist | 3 | | 10 | | 15 | |
| Tot Em–Vol | 3 | | 1 | | 8 | |
| Tot Ment St Terms | 6 | | 11 | | 23 | |

Legend: persuasive text (PT); explanations/counter arguments (Exp/C.Arg); epistemic terms (Epist); emotional–volitional terms (Em–Vol); mental state terms (Ment St terms).

To compare G's performance in the last two PTs to the performance of the controls in the same PTs we applied Crawford and Howell's [26] method, used to compare an individual with control samples that have modest N (e.g., <10). According to this method, the statistics of the control sample are treated as sample statistics, rather than as population parameters, and the *t*-distribution (with *n*—1 degrees of freedom) is used, rather than the standard normal distribution, to evaluate the abnormality of the individual's scores. In this modified *t*-test procedure, the p value represents the probability of individuals in the population from which the normative sample was drawn of obtaining a score as low as that observed for the individual.

Crawford and Howell's [26] method was applied to all the measures described in Table 2 (*t* values and two-tailed probabilities are reported in brackets). As the standard deviation of topic and ending was zero in the control group, it was impossible to perform the comparison with G's scores. For all the other measures, no significant differences were found.

**Table 2.** Comparisons between G and control scores.

| | G's Scores (*z*-Scores) | Controls' Mean (SD) | *t* | *p* |
|---|---|---|---|---|
| Reasons | 7 (−1.68) | 9.13 (1.27) | −1.58 | 0.16 |
| Exp/C.Arg. | 9 (−0.33) | 9.50 (1.50) | −0.31 | 0.76 |
| Total | 20 (−1.07) | 22.63 (2.45) | −1.01 | 0.34 |
| Reasons' levels | 18 (−1.79) | 25.00 (3.91) | −1.69 | 0.14 |
| Exp/C.Arg levels | 20 (−1.51) | 27.63 (5.05) | −1.42 | 0.20 |
| Epistemic terms | 15 (−0.69) | 18.75 (5.40) | −0.66 | 0.53 |
| Em–Vol terms | 8 (−1.65) | 17.50 (5.74) | −1.56 | 0.16 |
| Total | 23 (−1.36) | 36.25 (9.72) | −1.29 | 0.24 |

## 4. Discussion

In this article, we described an intervention implemented with a 13.2-year-old boy with ASD, G, without intellectual disability, aimed at improving his ability to compose persuasive texts, a pragmatic–linguistic ability that was clearly poor according to his teachers. This weakness was particularly striking in light of G's erudite language and cultivated comments. His refined references to political institutions, presented as possible methods of interpreting very common social interactions, made his discourse difficult to understand, especially to his peers.

Our design included an initial assessment (baseline phase), an intermediate assessment after two weeks, a six-session intervention phase, and a post-intervention assessment. The intervention drew on Asaro-Saddler and Bak's study [15], where Self-Regulated Strategy Development [16,17] was applied to enhance the writing of PTs. In our study, the POW + TREE intervention program was implemented in six sessions, subdivided into four phases: modeling, joint writing, guided writing and autonomous writing. To analyze the six texts considered in this study, three types of measures were used by two raters at baseline, intermediate and post-test time: (a) the presence/absence of the TREE components; (b) the quality of the reasons and explanations for these reasons and/or counter arguments; (c) the number of mental state terms.

The score assessing the presence of the TREE components increased from five to six from the baseline to the intermediate phase and then, quite remarkably, up to 20 in the post-test. A similar trend was attested in the growth of the quality of both reasons and explanations. Reasoning scores increased from two to four from the baseline to the intermediate phase and then, abruptly, to 18 at post-test, while explanations/counter argument scores increased from zero to 5 from the baseline to the intermediate phase, and then to 20 in the post-test. We believe the first increase might be attributed to the topic of PT4, centered on the use of the mobile phone, a particularly attractive one for G. In general terms, the nature of the contents most probably influenced the overall performance. However, this factor alone can hardly account for the transition from the initial texts, where the TREE structures were nearly absent, to the texts at the end of the intervention where these structures were very salient. In addition, we could observe a noticeable growth in the use of mental state terms, mainly on the epistemic side, i.e., from six words and expressions at the baseline, to 11 at the intermediate phase, to 23 in the post-test. It must be noted that, before the intervention, G's mental state terminology was present but unevenly distributed (see PT3 vs. PT4), while, at post-test, it became both richer and better distributed (PT5 and PT6).

If we consider the balance between the structural aspects of the texts in terms of the presence of the TREE components, and the qualitative aspects represented by mental state terms, we can better understand the nature of G's growth. In the very first text (PT1), the TREE structure is partial and mental state terms are rather poor and are only constituted by the use of epistemic words. At the other extreme, in PT6, the TREE structure is not only complete, but is also based on high-level

explanations, and, concomitantly, the mental state terminology reaches its maximum, with a balance between epistemic and emotional–volitional expressions.

What deserves attention, in our view, is that these improvements in both structural and lexical aspects match a psychological shift, from a writer-based- to a reader-based perspective, following Brown and colleagues [11]. In other words, G showed good linguistic resources in his first persuasive text, but he did not use these resources to persuade a hypothetical interlocutor. At the end of the intervention, G put these resources at the service of a reasonment based on representations, his own and the others'. It is probable that the above psychological shift was provoked at a precise phase of the intervention, namely the joint writing phase. For the first time, G had to compare his point of view with that of hypothetical others, with an argumentative aim in mind and, to this end, he had to choose well-focused words. At the same time, the joint writing practice paved the way to the autonomous writing phase, where G was stimulated to produce texts on his own, and apply all the devices he had been taught. This gradual transition from joint writing to G's autonomous production marks the transition from hetero- to self-regulation.

The following example (PT5, reactions after a dog bite), illustrates G's ability to analyze the different facets of the same issue and ponder the validity of arguments and counter-arguments. " . . . . . Even after a dog's bite, one can approach the animal world again (Topic). Not every animal behaves like dogs (R1). It has been a very rare accident (R2). The animal world has much to offer" (R3). Visibly, G provided a skilled argument and counter argument of the topic: (a) there are different categories of animals, which may differ in behavior; (b) a bite is not, per se, an absolute event and thus it cannot be generalized as a bad behavior; (c) animals are also capable of highly valuable behaviors. In addition, G supported his reasons with very appropriate explanations: "For instance, a tortoise or a rabbit are much less aggressive than a dog (in relation to R1). Moreover, dogs sometimes bite while playing (in relation to R2). People do love their pets because they offer them strong emotions: tenderness, love, friendship" (in relation to R3). On conceptual grounds, we must note how acute G's counter arguments are and, linguistically, how finely he can modulate his thoughts. In addition, we must remark that these conceptual and linguistic means serve a socio-cognitive function: the hypothetical friend's point of view is reverted into a convincing new perspective. Finally, in the ending, G recapitulated his argument in a very elegant register: "Therefore, even if you had an unpleasant accident, you should give a *second chance* (our italics) to the animal world!". Therefore, G included the act of biting in the broader category of an "accident", and categorized the invitation to try another approach with animals in more abstract terms, namely those of a "second chance", which he underlines with a significant exclamation mark.

Comparing G's performance in the post-test with his peers' produced text showed no significant differences. Therefore, G's best performance was close to that of his typically developing peers, at least on quantitative terms. Nevertheless, on qualitative grounds, we cannot help noticing that the controls organized both the initial presentation of the topic and the final recapitulation of the arguments in a more elaborate way. These children would characterize the very scenario of the hypothetical dialogue in detail, so as to render the whole argumentation process more plausible. In the ending, they would recapitulate more systematically the pros and cons, arguments and counter arguments with explicit reference to the "other's point of view". Another aspect that deserves attention is the extensive use of rhetorical devices—in particular, metaphors, idioms, proverbs and humor [27,28]—generally reported as a weakness in some individuals with ASD [27–31]. For example, in PT6, (topic: going to fast food restaurants), we found the following examples from different participants of the control group. "Non è tutto rose e fiori" (English: "It's not all fun and games") (before introducing a counter-argument). " . . . riempiono (i fast-food) di felicità le papille gustative" ( . . . "they (fast-foods) fill taste buds with happiness" (this is a totally unconventional metaphorical usage in Italian)). "Affogare la fame con patatine ed hamburger" ("To drown hunger with hamburger and chips" (also a totally unconventional metaphorical usage in Italian)). Both these metaphors are totally unconventional in Italian. Lastly, we will mention the frequency of explanations based on thoughts, opinions, and mental states. Ex:

"Going to fast-foods; to get free from thoughts . . . " " . . . to get relaxed and distract oneself"; "There you can exchange ideas with your friends, which will help you taking important decisions"; " . . . to take your mind off".

We believe this study presents some strengths. The teaching of POW + TREE followed a rigorous methodology, which was, however, flexibly implemented according to G's reactions, phase by phase. A short, but well-articulated, intervention let written argumentative abilities emerge in a child who was perceived by his teachers as particularly poor in this type of writing. We must also point to some methodological weaknesses and possible perspectives for future research. First of all, although we could rely on teachers' evaluations, we made no initial assessment of the controls' capabilities. Secondly, follow-up testing should be applied to check the solidity of the results obtained by G. Thirdly, a future step of the present study could focus on G's capability of producing other persuasive texts in the school context in order to check the generalizability of the outcomes obtained at the end of the intervention described here. Finally, we could consider the whole range of persuasive texts produced during the same lapse of time in both an individual child treated with the same type of intervention as G, and a control group. This would allow us to better grasp, beyond overall outcomes, the different trajectories of subjects with ASD, and those of typically developing children in completing this type of task. Although a case study based on a child with the characteristics of G cannot reflect the heterogeneous world of all individuals with ASD without intellectual disability, we believe it can shed light on the potentialities offered by the type of intervention described here.

## 5. Conclusions

In our study, we highlighted that writing a persuasive text involves abilities that go far beyond an academic task because they presuppose and, at the same time, stimulate the capability to think about the other's point of view in relation to one's own. We believe this capability represents an important form of reciprocity that can improve the subject's adaptive functioning.

**Author Contributions:** Conceptualization: S.M.; M.A.P.; methodology: S.M.; M.A.P.; software: T.G.S.; investigation: A.R.; data curation: S.M.; writing—original draft preparation, S.M.; M.A.P.; writing—review and editing, S.M.; M.A.P.; supervision: S.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

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brain sciences

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*Brief Report*

# How Attention to Faces and Objects Changes Over Time in Toddlers with Autism Spectrum Disorders: Preliminary Evidence from An Eye Tracking Study

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Received: 29 October 2019; Accepted: 26 November 2019; Published: 27 November 2019

**Abstract:** Further understanding of the longitudinal changes in visual pattern of toddlers with autism spectrum disorders (ASDs) is needed. We examined twelve 19 to 33-month-old toddlers at their first diagnosis (mean age: 25.1 months) and after six months (mean age: 31.7 months) during two initiating joint attention (IJA) tasks using eye tracking. Results were compared with the performance of age-matched typically developing (TD) toddlers evaluated at a single time-point. Autistic toddlers showed longitudinal changes in the visual sensory processing of the IJA tasks, approaching TD performance with an improvement in the ability to disengage and to explore the global space. Findings suggest the use of eye tracking technology as an objective, non-intrusive, adjunctive tool to measure outcomes in toddlers with ASD.

**Keywords:** autism spectrum disorders; toddlers; eye tracking; joint attention; longitudinal

## 1. Introduction

Autism spectrum disorders (ASDs) are neurodevelopmental conditions, affecting approximately 1% of children in Italy [1], and characterized by persistent deficits in social communication and interaction, along with the presence of restrictive and repetitive behaviors [2]. Eye tracking is a technique that is opening new avenues for quantitative, objective, simple, non-invasive evaluation of the visual patterns in young individuals with ASD [3,4]. In particular, it can be used to explore ASD atypicalities in visual social attention [5], the behavior of allocating attentional resources to social stimuli [6], and an area in which deficits have been well documented in individuals with ASD [7–9]. A more advanced form of social attention is joint attention (JA), which is the ability to coordinate visual attention with another individual to an object or event that emerges between 6 and 12 months of age in typical development [10]. Two types of JA are described in the literature: (1) Response to joint attention (RJA), which is the ability to follow the direction of other's gaze; and (2) initiating joint attention (IJA), which is the ability to use gaze to direct the attention of others towards a shared object

or event of interest [11]. JA impairment is consistently reported as one of the earliest and specific signs of ASD that becomes apparent at the end of the first year of life [12–15].

Eye tracking is a method that enables high-precision detection and accuracy characterization of the subtle variations in the spontaneous viewing patterns of JA in individuals with ASD [16,17]. Since it does not require advanced motor responses or language skills, eye tracking can offer useful insights when studying infants and toddlers with ASD [18,19].

Through a previous eye tracking study, we analyzed RJA and IJA in toddlers with ASD [20]. Results indicated different visual patterns between ASD and typically developing (TD) toddlers in IJA only. Specifically, toddlers with ASD looked longer at faces and had more transitions from the target object to the face, while TD toddlers looked more at the non-target object, and had more transitions from the non-target object to the face or from one object to another. These counterintuitive findings have been discussed in relation to the impairment in disengagement from face and in divided attention, which might compromise the ability to track more than one object on the scene.

On the basis of this previous investigation, in the current paper, we aimed to evaluate possible longitudinal changes of the visual pattern during the same IJA tasks in toddlers with ASD. To accomplish this aim, the same IJA tasks of our previous study were administered longitudinally, with an interval of six months. In particular, we focused on testing whether changes in the visual pattern of toddlers with ASD were following a developmental trajectory similar to that identified in typical development. Finally, we aimed to explore whether some clinical measures were predictive of visual pattern changes in toddlers with ASD.

## 2. Method

### *2.1. Participants*

Twelve toddlers with ASD and 15 age- and gender-matched TD toddlers participated in the study (Table 1). The sample of toddlers with ASD only partially (six subjects) overlaps that of our previous study [20], while the sample of TD is the same. The clinical diagnosis of ASD was established according to DSM-5 criteria [2], and confirmed by using algorithm cutoffs on the Autism Diagnostic Observation Schedule (ADOS-2) [21]. Exclusion and inclusion criteria were presented elsewhere [18]. All children (ASD and TD) received a non-verbal developmental evaluation through the administration of the performance subscale of the Griffiths Mental Developmental Scales (GMDS) [22]. The adaptive behavior profile of children with ASD was measured by means of the Vineland-II, a semi-structured interview with the individual's caregiver [23]. Control toddlers were typically developing according to parental report, and did not have any medical or developmental diagnoses. Typical development was also confirmed by the Child Behavior Check List 1.5–5 (CBCL) questionnaire [24]. All toddlers in the TD group scored below the borderline/clinical range (Table 1).

**Table 1.** Participants characteristics.

| | **ASD T1** $n = 12$ | **ASD T2** $n = 12$ | **TD** $n = 15$ | **ASD T1 vs. ASD T2** *p*-**Value** | **ASD T1 vs. TD T1** *p*-**Value** | **ASD T2 vs. TD T1** *p*-**Value** |
|---|---|---|---|---|---|---|
| | M (SD) | M (SD) | M (SD) | | | |
| Age (months) | 25.1 (4.6) | 31.7 (4.7) | 26.5 (4.1) | – | $t(24) = 0.86$, $p = 0.40$ | – |
| Gender: M, F | 10, 2 | 10, 2 | 13, 2 | – | $\chi^2 = 0.06$, $p = 0.81$ | – |
| ADOS-2, total | 14.9 (4.5) | 11.0 (3.7) | – | $t(22) = -2.31$, $p = 0.03$ | – | – |
| GMDS, performance | 74.9 (25.0) | 83.5 (13.6) | 102.5 (11.7) | $t(11) = -1.73$, $p = 0.11$ | $t(24) = 3.79$, $p = 0.001$ | $t(24) = 3.88$, $p = 0.001$ |
| Vineland-II, total | 75.8 (4.4) | 79.6 (15.9) | – | $t(4) = -0.86$, $p = 0.43$ | – | – |

ASD: Autism spectrum disorders; TD: Typically developing; M: Mean; SD: Standard deviation; ADOS: Autism Diagnostic Observation Schedule; GMDS: Griffiths Mental Developmental Scales.

ADOS-2 items belonging to the joint attention factor (pointing, gesture, showing, initiation of joint attention, unusual eye contact) were chosen as measures of JA [25].

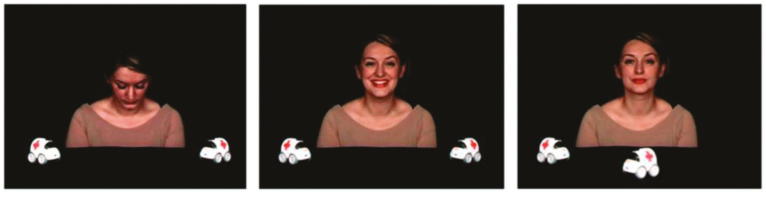
All parents provided written informed consent, including permission to use the video recordings for scientific reasons. The experimental procedures and the informed consent were approved by the Institutional Review Board of the Clinical Research Institute for Child and Adolescent Neurology and Psychiatry.

### *2.2. Procedure and Stimuli*

Toddlers with ASD were assessed at their first diagnosis—time 1 (T1) (mean age, 25.1 months; SD, 4.6 months; age range, 19–33 months; and after six months—time 2 (T2) (mean age, 31.7 months; SD, 4.7 months). The comparison group of TD toddlers was assessed only at the first time point (T1: Mean age, 26.5 months; SD, 4.1 months; age range: 18–30 months).

Eye tracking data were acquired using an SMI-RED 500 Eye Tracker (SMI, SensoMotoric Instruments, Teltow, Germany). Both eyes were tracked with a rated accuracy < 1° and a sampling frequency of 120 Hz, which is a sufficient sampling frequency rate to detect two-point data according to an authoritative study [26]. The toddlers sat on a child chair, approximately 50 cm from the monitor, in front of a small table. No explicit instructions were given. The experiment started with a 5-point calibration sequence, in which a cartoon was used as calibration point to catch the toddlers' attention to the screen. The calibration was repeated until the deviation from the known calibration target for both the x and y components was below 2°.

The IJA paradigm consisted of two different tasks: (1) IJA task with a predictable event (IJA-1): A female model was positioned between two little cars placed on the table in front of her, and one of the two cars ('target object') moved, while the actor maintained a direct gaze to the child with a neutral expression; (2) IJA with an unpredictable event (IJA-2): The same female actor was initially alone in the scene, and then a toy truck ("target object") appeared unexpectedly from outside of the scene and crossed the screen while the actor maintained a direct gaze with a neutral expression. Each task included three phases: (a) Looking down (2 s); (b) smiling (2 s); (c) JA (7 s) (Figure 1 includes screenshot of the video). Four trials per conditions were presented to each child. Each trial was preceded by a colorful "attention-getter" that was displayed at the center of the screen until the toddler looked at it for at least 500 ms. The total duration of the eye tracking session was, on average, two min (the duration varied slightly from one child to another, according to their ability of performing the calibration and of looking at the attention-getter).



(**a**)

(**b**)

**Figure 1.** Initiating joint attention (IJA) task. (**a**) IJA task with a predictable event (IJA-1); (**b**) IJA task with an unpredictable event (IJA-2).

### *2.3. Data Analysis*

Measures of JA were calculated on the JA segments of the tasks. Measures referred to transitions and were computed by extracting raw data and analyzing them in Matlab (MathWorks, Natick, MA, USA) using homemade scripts. Specifically, we evaluated the number of transitions from face to target object and the number of transitions from objects to face (as an indication of the alternating looking pattern between them). In IJA-1, we also computed between-object transitions and the normalized transition score (that is, the difference between the total number of transitions from target object to face and the total number of transitions from non-target object to face divided by the total number of transitions from either object to face).

In addition, we selected the following areas of interest (AOIs): Model's face, target object, and non-target object (the object that did not move in the IJA-1 task). For each of these AOIs, we calculated fixation duration (FD), computed as a percentage of the total (i.e., FD on that AOI relative to the participants' on-trial FD). A fixation threshold of 60 ms was applied.

### *2.4. Statistical Analysis*

Statistical analysis was completed using SPSS 20 software for Mac (SPSS, Chicago, IL, USA). Descriptive analyses for the continuous variables (means and standard deviations) and ordinal variables (frequencies and percentages) were performed on the demographic and clinical variables. Normality of the data was evaluated using the Shapiro–Wilk test and the equality of the variances with Mauchly's sphericity test.

For the inferential analyses, three tests were performed: (1) One-way ANCOVAs to evaluate differences at T1 in the visual pattern between ASD toddlers and TD, using developmental level as a covariate; (2) a repeated measures ANCOVA (T1 versus T2) for ASD, to evaluate changes on clinical and eye tracking measures using the difference between age at T1 and age at T2 as a covariate; (3) one-way ANCOVAs to evaluate differences in the visual pattern between toddlers with ASD at T2 and the visual pattern of TD toddlers at T1, using developmental level and age as covariates. The significance threshold for all tests was set at 0.05 after Bonferroni correction. Effect sizes were estimated by partial eta squared ($\eta^2$).

A stepwise linear regression was performed to identify T1 clinical measures predicting eye tracking performance at T2. Associations between eye tracking and clinical measures at T2 were examined using Spearman's correlations. In addition, in order to evaluate whether modifications in eye tracking pattern were associated with modifications in social functioning, we compared clinical measures (ADOS items measuring the "joint attention factor" [27] and Vineland-II items [23]) at T1 and T2, using paired-sample *t*-tests.

## 3. Results

Results of the eye tracking measures are reported in Table 2.

**Table 2.** Eye tracking measures.

| | ASD T1 $n = 12$ Mean (SD) | ASD T2 $n = 12$ Mean (SD) | TD $n = 14$ Mean (SD) | ASD T1 vs. ASD T2 $p$-Value | ASD T1 vs. TD T1 $p$-Value | ASD T2 vs. TD T1 $p$-Value |
|---|---|---|---|---|---|---|
| Initiating JA-1 | | | | | | |
| FD: Face | 24.85 (20.11) | 19.34 (17.06) | 20.14 (14.61) | F = 0.86, $p$ = 0.36, $\eta^2$ = 0.03 | F = 0.53, $p$ = 0.47, $\eta^2$ = 0.02 | F = 0.07, $p$ = 0.79, $\eta^2$ = 0.03 |
| FD: Target object | 22.15 (13.69) | 34.54 (23.16) | 38.62 (20.62) | F = 2.17, $p$ = 0.17, $\eta^2$ = 0.18 | F = 2.22, $p$ = 0.15, $\eta^2$ = 0.08 | F = 2.15, $p$ = 0.16, $\eta^2$ = 0.08 |
| FD: NT object | 3.84 (3.51) | 8.48 (5.11) | 13.56 (9.92) | F = 4.37, **$p$ = 0.038** *, $\eta^2$ = 0.537 | F = 6.69, **$p$ = 0.016** *, $\eta^2$ = 0.218 | F = 1.29, $p$ = 0.27, $\eta^2$ =0.05 |
| T to face from target object | 5.15 (4.11) | 4.58 (2.87) | 1.42 (1.13) | F = 0.89, $p$ = 0.77, $\eta^2$ = 0.01 | F = 5.781, **$p$ = 0.026** *, $\eta^2$ = 0.216 | F = 2.48, $p$ = 0.12, $\eta^2$ = 0.09 |
| T to face from NT object | 0.40 (0.52) | 0.84 (0.56) | 1.38 (1.19) | F = 6.29, **$p$ = 0.024** *, $\eta^2$ = 0.711 | F = 5.55, **$p$ = 0.029** *, $\eta^2$ = 0.217 | F = 2.22, $p$ = 0.18, $\eta^2$ = 0.07 |
| Normalized transition score | 0.84 (0.20) | 0.77 (0.27) | 0.40 (0.48) | F = 0.80, $p$ = 0.39, $\eta^2$ = 0.08 | F = 4.99, **$p$ = 0.036** *, $\eta^2$ = 0.185 | F = 3.49, $p$ = 0.07, $\eta^2$ = 0.132 |
| T from face to target object | 4.08 (2.27) | 4.75 (2.73) | 3.27 (2.08) | F = 1.08, $p$ = 0.25, $\eta^2$ = 0.13 | F = 0.99, $p$ = 0.33, $\eta^2$ = 0.04 | F = 0.64, $p$ = 0.43, $\eta^2$ = 0.03 |
| T from face to NT object | 0.83 (1.03) | 1.33 (1.43) | 1.33 (1.44) | F = 5.67, **$p$ = 0.04** *, $\eta^2$ = 0.36 | F = 1.07, $p$ = 0.31, $\eta^2$ = 0.04 | F = 0.35, $p$ = 0.56, $\eta^2$ = 0.02 |
| Between object transitions | 3.89 (3.43) | 4.42 (3.11) | 5.07 (3.91) | F = 4.55, $p$ = 0.06, $\eta^2$ = 0.29 | F = 0.21, $p$ = 0.15, $\eta^2$ = 0.08 | F = 2.08, $p$ = 0.16, $\eta^2$ = 0.08 |
| Initiating JA-2 | | | | | | |
| FD: Face | 37.57 (27.03) | 13.65 (17.52) | 16.76 (16.86) | F = 6.00, **$p$ = 0.03** *, $\eta^2$ = 0.375 | F = 8.02, **$p$ = 0.01** *, $\eta^2$ = 0.276 | F = 0.001, $p$ = 0.97, $\eta^2$ = 0.0001 |
| FD: Target object | 28.52 (22.12) | 34.54 (23.16) | 31.84 (22.30) | F = 0.62, $p$ = 0.45, $\eta^2$ = 0.06 | F = 0.07, $p$ = 0.79, $\eta^2$ = 0.003 | F = 0.12, $p$ = 0.73, $\eta^2$ = 0.006 |
| T from target object to face | 4.42 (1.78) | 4.00 (1.79) | 1.71 (1.37) | F = 1.93, $p$ = 0.19, $\eta^2$ = 0.18 | F = 7.65, **$p$ = 0.011** *, $\eta^2$ = 0.242 | F = 6.21, $p$ = 0.15, $\eta^2$ = 0.20 |
| T from face to target object | 4.0 (2.0) | 4.79 (2.75) | 1.31 (1.37) | F = 2.62, $p$ = 0.14, $\eta^2$ = 0.23 | F = 7.47, **$p$ = 0.012** *, $\eta^2$ = 0.237 | F = 4.57, **$p$ = 0.04** *, $\eta^2$ = 0.222 |

FD: fixation duration; T: transitions; NT: non-target object. * Significant at 0.05 after Bonferroni correction. All significant $p$-values are reported in bold.

### *3.1. ASD and TD Comparison at T1*

In IJA-1, toddlers with ASD had significantly higher transitions from target object to face ($p$ = 0.026), and significantly higher normalized transition scores ($p$ = 0.036) compared to TD. Conversely, TD toddlers made significantly higher transitions from non-target object to face ($p$ = 0.029), and had higher fixations to the non-target object ($p$ = 0.016) than toddlers with ASD. No other significant differences were detected. In the IJA-2 task, toddlers with ASD had both significant higher transitions from target object to face ($p$ = 0.011) and from face to target object ($p$ = 0.012) than TD toddlers. Moreover, toddlers with ASD had significant higher FD to face ($p$ = 0.01).

### *3.2. Longitudinal Changes in ASD and Comparison with TD*

In the IJA-1 task, toddlers with ASD showed a significant increase in transitions from non-target object to face with time ($p$ = 0.024), so that at T2, no significant difference was still present between ASD and TD (Figure 2a). In IJA-1, toddlers with ASD also showed a significant increase ($p$ = 0.04) of

transitions from face to non-target object (Figure 2b). In addition, there was a significant increase with time of FD to the non-target object ($p = 0.038$), so that at T2, no significant difference was still present between ASD and TD (Figure 2c).

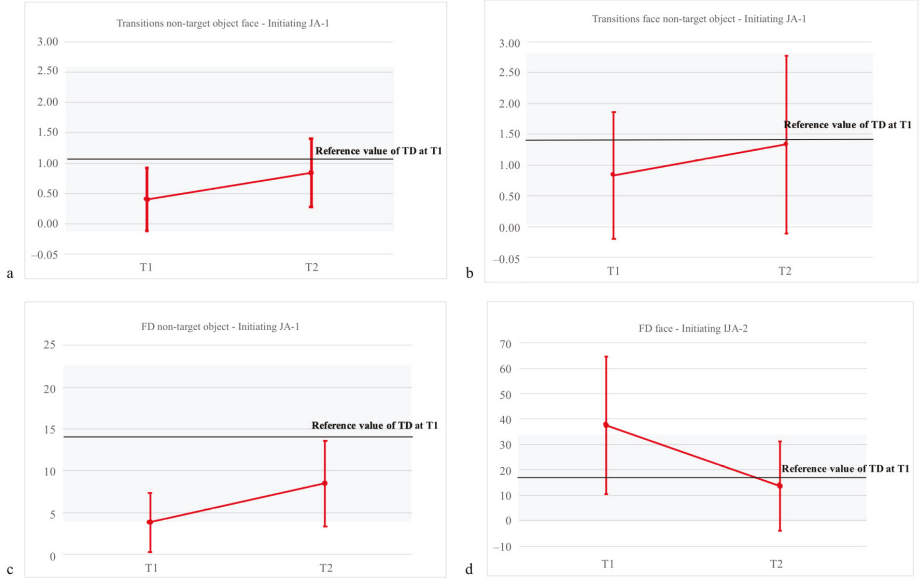



**Figure 2.** Significant longitudinal changes (with SD) in eye tracking measures in the ASD group. For the purpose of comparison, the reference values in the TD group are reported as a black line. (**a**) Change in transitions from non-target object to face in the Initiating JA-1 task; (**b**) change in transitions from face to non-target object in the Initiating JA-1 task; (**c**) change in fixation duration at non-target object in the Initiating JA-1 task; (**d**) change in fixation duration at face in the Initiating JA-2 task.

In the IJA-2 task, no significant effect of time for transitions was observed, but a significant decrease with time of FD to face was noticed ($p = 0.03$), so that at T2, no significant difference between ASD and TD was detected as far as FD for face is regarded (Figure 2d). Pairwise comparison showed that while transitions from face to target object were still higher at T2 in ASD compared to TD ($p = 0.04$), differences in transitions from target object to face disappeared at T2.

### *3.3. ADOS Predictors of Eye Tracking Performance at T2*

For the IJA-1 task, it was observed that ADOS_A7-Pointing at T1 was an independent predictor of transitions from face to non-target object at T2 ($\beta = -0.63$, adj-$R^2 = 0.34$, $p = 0.027$) (Figure 3a), and that ADOS_B9-Showing at T1 was an independent predictor of transitions from target object to face at T2 ($\beta = -0.64$, adj-$R^2 = 0.35$, $p = 0.025$) (Figure 3b).

Finally, for the IJA-2, it was observed that ADOS_A8-Gesturing at T1 was an independent predictor of FD at target object at T2 ($\beta = -0.64$, adj-$R^2 = 0.34$, $p = 0.035$) (Figure 3c).

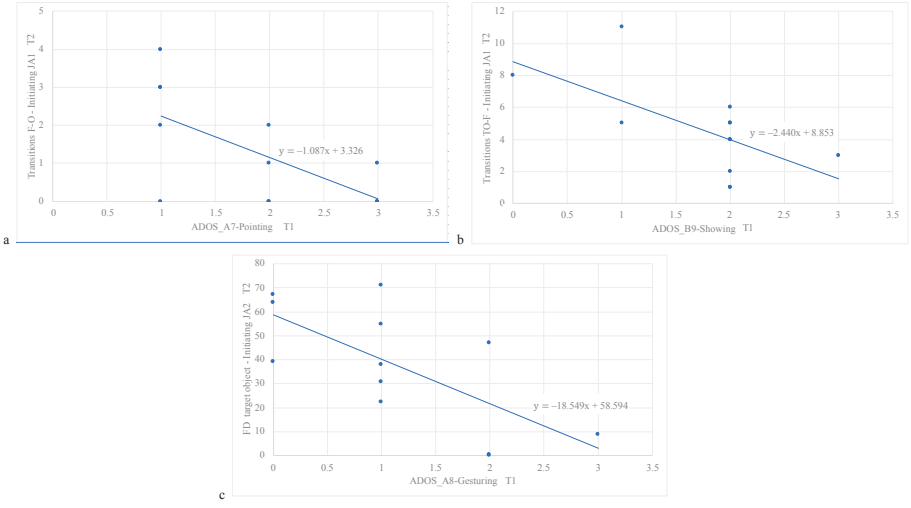



**Figure 3.** Significant clinical predictors at T1 for eye tracking measures at T2 in the ASD group. (**a**) ADOS_A7-Pointing at T1 as an independent predictor of transitions from face to non-target object at T2 in the IJA-1 task; (**b**) ADOS_B9-Showing at T1 as an independent predictor of transitions from target object to face at T2 in the IJA-1 task; (**c**) ADOS_A8-Gesturing at T1 as an independent predictor of fixation duration at target object at T2 in the IJA-2 task.

### *3.4. Correlations with Developmental Quotient*

The Performance developmental quotient at T1 was not a predictor of any change in eye tracking measure at T2. No significant correlation between GMDS-Performance or difference in GMDS-Performance between T2 and T1 and eye tracking measures were found in ASD at T2.

### *3.5. Longitudinal Modifications in Clinical Measures*

As regards ADOS items, ADOS-2_A8-Gesturing significantly changed from T1 to T2 (T1: 1.20 ± 0.91; T2: 0.50 ± 0.52; $p = 0.038$).

As far as the Vineland-II scores, significant modifications in the items "Receptive" (T1: 12.00 ± 3.39; T2: 20.40 ± 8.90; $p = 0.04$), "Expressive" (T1: 14.00 ± 9.94; T2: 23.20 ± 10.94; $p = 0.04$), and "Community" (T1: 4.60 ± 3.97; T2: 7.60 ± 4.33; $p = 0.039$) were observed.

## 4. Discussion

While confirming that toddlers with ASD show an atypical visual pattern for IJA compared to toddlers with TD, the findings of the present investigation support the hypothesis of early longitudinal changes in the visual pattern of toddlers with ASD toward a greater similarity to that characteristic of TD subjects. Over a period of six months, the visual pattern of ASD is no longer characterized by the prevalence of fixation to face and by the indifference to non-target object. Moreover, significantly more transitions from non-target object to face are observed.

These three modifications make the visual performance of toddlers with ASD in both IJA tasks very different from their previous performance at T1, and more similar to the performance of TD toddlers six months younger. Two mutually reinforcing factors can be called into question. First, we can hypothesize that a maturational process of the anatomical systems supporting JA occurred [28–30].

Second, we have to mention that all toddlers with ASD in our sample are engaged in some type of behavioral and or psycho-educational intervention, which may boost neuroplasticity [31]. In fact, JA constitutes a primary target for early ASD intervention [32–34], which in turn may have had a

positive impact on developmental trajectories of JA, as previously observed [35]. In this framework, a seminal randomized clinical trial detected that a group of ASD children that carried out a specific developmental behavioral intervention showed both neurotypical patterns of cortical activation and increased neural response to social stimuli [36]. Thus, based on our findings, we can speculate that IJA atypicalities detected in ASD toddlers at T1 represent delay rather than impairment, since they could improve over time.

In a previous study with older children with ASD [37], using an integrating eye tracking and electroencephalography approach, we reported trends of changes in both brain activity and connectivity in the JA circuits after a six-month rehabilitative intervention, which were correlated with modifications in gaze measures. Thus, we can also hypothesize that the longitudinal modifications observed in the present study are associated with a modification of neurophysiological mechanisms.

The global longitudinal changes in the visual sensory processing of our IJA tasks seem to be linked to the increasing of transitions and, in particular, of transitions to non-target object. We suppose that this increase of transitions is an expression of the improved abilities in attention disengagement and in the global space exploration, which represent two skills typically impaired in early ASD.

Indeed, a preference for local over global processing has been repeatedly indicated as a core feature of the autistic phenotype (e.g., [38,39]). Moreover, previous studies reported that infants later diagnosed with ASD were slower to disengage their attention from one object to another, compared to TD infants [18,40,41].

The increased ability of our toddlers with ASD to shift their attention from face to non-target object should therefore be interpreted as a positive sign for the development of IJA and, more broadly, social competencies [42]. Accordingly, the developmental changes in the visual sensory processing of the IJA tasks are related to improvements both in the social behaviors included in the "JA factor" of the ADOS-2 [27], and in specific items measuring Communication and Daily Living Skills of the Vineland-II [23]. This block of evidence makes the current eye tracking findings more robust from a translational point of view. Notably, the modifications we observed are independent from developmental quotient, i.e., they are not attributable to modifications in developmental skills from T1 to T2. Thus, repeated eye tracking evaluation may represent an objective and specific outcome measure in toddlers with ASD [43], since it is able to detect modifications in the visual pattern reflecting brain plasticity of the social brain.

Finally, we examined clinical predictors of modifications in eye tracking profile among toddlers with ASD, and we observed that low clinical measures of autism severity at some ADOS-2 items (i.e., pointing, showing, and gestures) were correlated with increased eye tracking longitudinal changes. This negative correlation indicates that a less severe clinical performance on these measures at an earlier age can be a good predictor for the approaching of the IJA visual pattern to that of TD. Previous studies correlated eye tracking data with clinical outcome. For example, a recent cross-sectional study of infant siblings has demonstrated that less gaze alternations between an interaction partner and an interesting event at 10 months was associated with more social impairment and less showing and pointing at 18 months [44]. In addition, different visual responses to dynamic social stimuli in toddlers with ASD have been linked to differences in autism severity and developmental functioning 1–2 years later [45].

Several limitations need to be acknowledged in this study. First, as this was a small sample investigation, replication of the initial finding is needed. Second, since this study did not include the eye tracking measure at T2 for the TD group, it is not possible to compare the pattern of eye gaze between ASD and control subjects in the longitudinal evaluation. Consequently, it remains to be elucidated whether differences between groups are present at T2 also, and/or whether new differences emerge. Despite these drawbacks, this study suggests changes in looking patterns of toddlers with ASD during a brief interval (i.e., six months) that results in IJA performance more similar to those of subjects with TD at T1. Our data do not allow disentangling the relative contributions of rehabilitative treatment and normal brain maturation on changes in eye tracking profile. Future large-scale randomized

controlled trials after standardized rehabilitative intervention are necessary before translating eye tracking evaluation into a treatment outcome measure to include in clinical practice.

**Availability of Data and Material:** The datasets generated and/or analyzed during the current study are not publicly available due to the privacy policy (containing information that could compromise research participant privacy/consent), but are available from the corresponding author on reasonable request and with permission of parents of the involved children.

**Author Contributions:** Conceptualization, A.N.; Formal Analysis, L.B.; Data Curation, M.B., C.L., V.C., M.T.; Patient recruitment: F.M., S.C., C.L.; Writing—Original Draft Preparation, L.B., F.M.; Writing—review and editing, L.B., F.M., A.N., S.C.; Supervision, F.M., C.C.; Project Writing, A.N., L.B.

**Funding:** This study was supported by the ALERT project, funded by the Italian Ministry of Health (CCM/2011).

**Acknowledgments:** The authors thank all infants and their parents that participated in this study. This work has been made possible thanks to the contribution of the ALERT Team, which consisted of Giulia Campatelli, Giulia Crifaci, Alessandra Darini, and Rosamaria Siracusano.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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ISBN 978-3-0365-0163-5