nutrients

Gluten-Related Disorders
Time to Move from Gut to Brain

Edited by
Nigel Hoggard, David S Sanders and Marios Hadjivassiliou

MDPI

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Preface to "Gluten-Related Disorders: Time to Move from Gut to Brain"

As guest editors of this theme, we aimed to concentrate on neurological aspects of coeliac disease (CD) and gluten sensitivity (GS), an increasingly important but poorly recognized area. The articles presented here cover a wide spectrum, including neuropathology, psychiatric manifestations, interactions with other extraintestinal manifestations (dermatitis herpetiformis), overlap between CD/GS and stiff person syndrome, what we know about autism and gluten sensitivity, and the increasingly important area of cognitive deficits in CD and GS. Furthermore, one article addresses the important issue of follow-up in the context of monitoring both clinically and serologically, highlighting that such an approach is essential in the prevention of permanent neurological disability. This collection also includes a neurophysiological study in an attempt to unravel the pathophysiology of neurological dysfunction in CD and GS. These articles build up on numerous previous publications on the subject, many by the editors and authors involved in this publication. This book should be used alongside previous publications that cover manifestations such as gluten ataxia, gluten neuropathy, and gluten encephalopathy. We hope that this book will stimulate interest in the field and encourage further research in an area that merits further consideration.

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Psychiatric Manifestations of Coeliac Disease, a Systematic Review and Meta-Analysis

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Abstract: Background: Coeliac disease (CD) is increasingly prevalent and is associated with both gastrointestinal (GI) and extra-intestinal manifestations. Psychiatric disorders are amongst extra-intestinal manifestations proposed. The relationship between CD and such psychiatric disorders is not well recognised or understood. Aim: The aim of this systematic review and meta-analysis was to provide a greater understanding of the existing evidence and theories surrounding psychiatric manifestations of CD. Methodology: An online literature search using PubMed was conducted, the prevalence data for both CD and psychiatric disorders was extracted from eligible articles. Meta analyses on odds ratios were also performed. Results: A total of 37 articles were included in this review. A significant increase in risk was detected for autistic spectrum disorder (OR 1.53, 95% CI 1.24–1.88, $p < 0.0001$), attention deficit hyperactivity disorder (OR 1.39, 95% CI 1.18–1.63, $p < 0.0001$), depression (OR 2.17, 95% CI 2.17–11.15, $p < 0.0001$), anxiety (OR 6.03, 95% CI 2.22–16.35, $p < 0.0001$), and eating disorders (OR 1.62, 95% CI 1.37–1.91, $p < 0.00001$) amongst the CD population compared to healthy controls. No significant differences were found for bipolar disorder (OR 2.35, 95% CI 2.29–19.21, $p = 0.43$) or schizophrenia (OR 0.46, 95% CI 0.02–10.18, $p = 0.62$). Conclusion: CD is associated with an increased risk of depression, anxiety, eating disorders as well as ASD and ADHD. More research is required to investigate specific biological explanations as well as any effect of gluten free diet.

Keywords: coeliac disease; gluten free diet; psychiatric manifestations; autistic spectrum disorder; attention deficit hyperactivity disorder; depression; anxiety; bipolar disorder; schizophrenia; eating disorders

1. Introduction

The prevalence of CD is 1% in the Western population and it is increasing amongst both pediatric and adult populations [1–3]. Possible explanations for this increase include easier diagnostic methods and better targeted screening [4,5]. In addition to classic gastrointestinal (GI) symptoms, extra-intestinal symptoms such as neurological, psychiatric, and skin related are increasingly recognised [1,6–9]. These extra-intestinal symptoms when presenting in isolation are challenging in the diagnosis of CD [1,10].

Psychiatric disorders often reported in the literature include autistic spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), bipolar disorder, depression, anxiety, schizophrenia, other psychotic disorders and eating disorders [1,7,8,10–15]. These psychiatric disorders are therefore the focus of this systematic review and meta-analysis.

Interaction between CD and Psychiatric Disorders

A complex interaction between CD and such psychiatric disorders is proposed in the literature [10,12,15,16]. Theories are often split into specific and non-specific mechanisms [16]. Specific mechanisms refer to biological processes that may be producing overlapping pathologies, such as speculation over a direct 'gut–brain' relationship [12,16,17]. Non-specific mechanisms include the social and emotional consequences of CD diagnosis [7].

A strict gluten-free diet (GFD) is the only effective treatment for CD and this is often claimed to influence the risk of psychiatric disorders, but the exact role of the GFD has not been investigated in detail [7,8,10]. Some propose that the improvement of the GI symptoms with GFD may be protective against the development of psychiatric disorders [1]. However, there are also claims that it may increase such risk due to the detrimental effect of GFD on quality of life [1,7]. Conversely, psychiatric disorders can hinder adherence to the GFD, suggesting a need for the appropriate treatment of psychiatric issues in order to improve overall outcomes [7].

The proposal of a direct gut–brain relationship contributing to the pathophysiology, commonly features in literature, in particular in reference to schizophrenia and ASD [1,12,16,18–21]. Theories often describe autoimmunity and inflammation as potentially playing a role [11,22,23]. Other theories highlight the fact that the gastrointestinal tract is the region of entry of many substances that may be implicated to psychiatric pathology [19]. Furthermore, the ingestion and breakdown of gluten into immunogenic peptides leaking through the intestinal wall and getting into the brain may potentially interfere with its functioning [12,13,19–21,24].

Endogenous essential amino acids, such as tryptophan are known to be crucial in the production of serotonin. Despite being located in the gut, serotonin also plays an important role in mood regulation and cognition, whilst enabling GI regulation [25]. For example, Groer et al., (2018) proposed that insufficient tryptophan levels are associated with obesity and inflammation and increase risk of maternal depression in obese pregnant women [26].

Dehhaghi, Kazemi Shariat Panahi, and Guillemin (2019) discuss evidence concerning molecular communications between microbiota within the gut and the CNS, explaining how poor integrity of the intestinal barrier contributes to poor CNS function, which has a subsequent influence on mood and behaviour.

Healthy gut microbiota is vital in the protection against both psychiatric disorders, as well as GI disorders such as CD [25]. Altered gut microbiota have been identified in individuals with CD, indicating this as a partial cause of inflammatory responses to gluten [27]. Sacchetti and Nardelli (2019) argue a relationship between gut microbiota and CD, however they also acknowledge that CD is a multifactorial disease and therefore this alone does not fully explain such manifestations. Evidence suggests that gut microbiota has the ability to also influence mood and behaviour, as it has been implicated in psychiatric disorders, such as anxiety and depression [25]. Individuals with depression have been found to possess different gut microbiota to those without [25]. There is therefore evidence of an important interaction between the brain and the gut that could potentially add to the pathophysiology of such extraintestinal manifestations.

Despite a long history of research investigating associations between CD and psychiatric disorders the literature is often conflicting, regularly concluding that there is limited knowledge and highlighting the need for further investigations [1,16,17,19,22,28–30]. Additionally, small sample studies limit the reliability and generalisability of these findings [28].

The aim of this systematic review and meta-analysis is to overview the existing literature on coeliac diseases and psychiatric disorders. Furthermore, we wanted to determine the prevalence each psychiatric disorder in patients with coeliac disease and vice versa in order to calculate the respective odds ratios to have on disorder when suffering from the other.

2. Materials and Methods

2.1. Literature Search Strategy

A systematic computer-based literature search using the PubMed database was conducted on the 14 May 2019. For the search, we used two Medical Subject Headings (MeSH) terms. Term A was "Celiac" or "Coeliac". Term B was "Psychiatric" or "Depression" or "Depressive" or "Psychosis" or "Psychotic" or "Schizophrenia" or "Schizoaffective" or "Anxiety" or "Mood disorder" or "Mood disorders" or "Autism" or "Autistic" or "Asperger" or "Asperger's" or "Anorexia" or "Anorexic" or "Bulimia" or "Bulimic" or "Eating disorder" or "Eating disorders" or "Bipolar" or "Manic" or "Mania" or "Hypomanic" or "Hypomania" or "ADHD" or "Attention Deficit Hyperactivity disorder" or "PTSD" or "Stress disorder". Three filters were applied; English language, human participants, and full text availability. We also perused the reference lists of the papers so as to try and include further relevant paper that were not identified with the above-mentioned search strategy.

2.2. Inclusion and Exclusion Criteria

1. Articles needed to provide original data.
2. Articles needed to concern the relationship of CD and psychiatric disorders.
3. CD should have been confirmed, either serologically with anti-endomysial (EMA), or a duodenum biopsy.
4. Formal diagnosis of psychiatric disorders should have been made.

All articles were abstract screened by a minimum of three authors in a blinded fashion using Rayyan software to ensure accuracy. Those found to meet any of the exclusion criteria were removed and any conflicts were settled by consensus during a face-to-face meeting in which the abstracts were reread. All remaining papers were screened again as a full article by at least two authors and conflicts were settled as before. Where a paper was not available online, a university interlibrary request was made for the item, a British Library request and failing these we attempted to find the authors contact details.

Figure 1 contains a Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow chart displaying this process.

2.3. Statistical Analyses

A database was developed using IBM SPSS Statistics (version 23.0 for Mac). Data was extracted from each study and included: study type, population size, type of psychiatric disorder, prevalence of the psychiatric disorder, whether this concerned an adult or pediatric population, and information about GFD. Frequencies and descriptive statistics were examined for each variable. The outcomes of interest were the proportion of patients with CD suffering from each psychiatric disorder and the proportion of patients suffering from each psychiatric disorder that had CD.

The meta-analysis of odds ratios was conducted using the RevMan program (RevMan, 2014) as suggested by the Cochrane Collaboration Group. Heterogeneity between studies was assessed using the I2 statistic. Data were analysed using a random effects model.

A value of $p < 0.05$ was considered to be statistically significant.

2.4. Compliance with Ethical Guidelines

This article is based upon previously published studies. The article follows the journal's ethical guidelines.
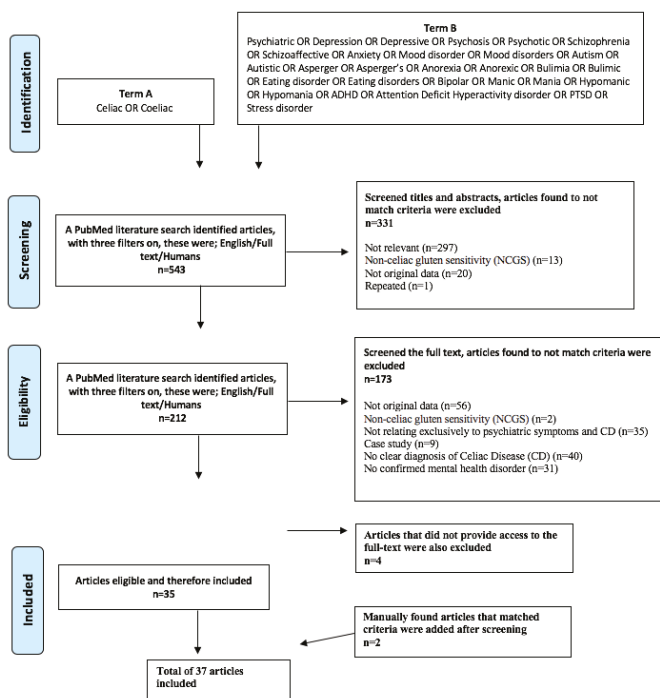
Figure 1. PRISMA flow chart displaying this selection process.

3. Results

A total of 543 articles were identified following this search, 331 were excluded due to not matching criteria based on titles and abstracts alone. A second screening of the full-text on the remaining 212 resulted in 173 articles being excluded. A further four was excluded due to not providing the full text, leaving 35 articles eligible to be included in this review. Another two articles were manually found during this screening process that also fitted the criteria. Therefore, a total of 37 articles that matched inclusion criteria were identified to be included in this review (Figure 1). Table 1 represents a summary of the descriptive characteristics of these studies included in this review.

The 37 articles were categorised according to specific psychiatric disorders and these included autistic spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD), schizophrenia or other psychotic disorders, depression, anxiety, bipolar disorder, and eating disorders. Articles were then analysed according to the prevalence of these psychiatric disorders in patients with CD and vice versa. However, no articles investigated CD amongst patients with depression, anxiety, bipolar disorder, or schizophrenia, therefore the pooled prevalence of CD within these disorders was not calculated. Additionally, a total of 15 articles also investigated the role of the GFD in such disorders and these findings were also examined.

3.1. ASD and ADHD

ASD literature consisted of nine articles in total and comprised of 39,207 participants [31–39]. Only one found statistically significant findings, therefore only one concluded the need for routine CD screening within the ASD population [31].

Table 1. Characteristics of studies included in this review

Parameter	Value
Number of papers	37
Population (%)	
Adult	32
Children	46
Mixed	22
Type of study	
Cohort	2
Case-controlled	18
Cohort and Case-controlled	1
Cross-sectional	14
Psychiatric disorder	
ASD	9
ADHD	8
Mood disorders	20
Schizophrenia and other psychotic disorders	6
Eating disorders	9
Year of publication (%)	
Until 2000	5
2000–2009	43
2010–2019	51

This included 38,440 participants with a diagnosis of CD. In total, 3336 were found to have ASD making the pooled prevalence of ASD in CD 8.7%. Such information about the prevalence of ASD in CD was available through two cross-sectional [32,33] and four case-controlled studies [34–37]. The meta-analysis of the four case-controlled studies is summarized in a forest plot in Figure 2a, the odds of having ASD was significantly higher in the CD groups compared to controls (OR 1.53, 95% CI 1.24–1.88, $p < 0.0001$). Figure 2b shows a funnel plot in which presents heterogeneity in the studies included.

(**a**)

(**b**)
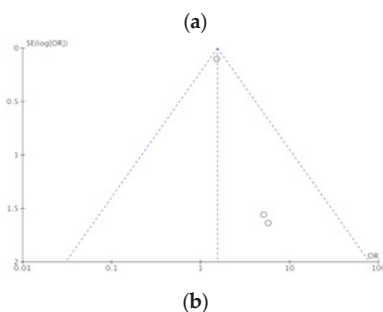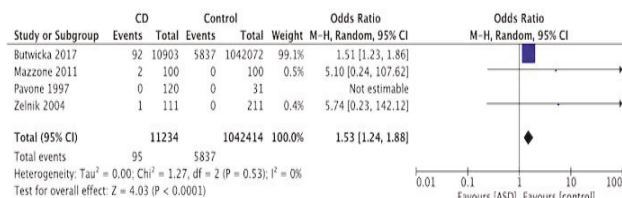
Figure 2. (**a**) Forest plot of pooled prevalence of ASD in CD. (**b**) Funnel plot investigating distribution in ASD studies.

Investigation into the prevalence of CD in patients with ASD was done by three cross sectional studies [33,38,39] and two case-controlled studies [31,37]. Of the 767 ASD participants, ten were found to have CD making the pooled prevalence of CD in ASD 1.3%. All of these individuals came from the same study, which is the only one that confirmed significant findings [31]. In addition to this, Juncia et al. (2018) noted GI symptoms in 34% of a pediatric ASD sample. Józefczuk et al. (2018) found no difference between the presence of CD-specific antibodies in ASD patients and controls, or any deficits in intestinal permeability.

Out of eight articles on ADHD, two concluded a significant association between ADHD and CD [34,40]. One of these referred to a sample size of eight participants of which two (siblings) were found to have ADHD as an initial presentation of CD [40]. The eight articles included a total of 12,366 participants. The prevalence of ADHD in CD was assessed by one case series study [41] two cross sectional studies [42,43] two case-controlled studies [34,44] and one cohort study [40]. Out of 11,965 CD participants, 165 were found to have ADHD resulting in a pooled prevalence of ADHD in CD of 1.4%. The meta-analysis of the two case-controlled studies is summarized in a forest plot in Figure 3a, the odds of having ADHD was significantly higher in the CD groups compared to controls (OR 1.39, 95% CI 1.18–1.63, $p < 0.0001$). Figure 3b shows a funnel plot for these studies. The prevalence of CD in ADHD was investigated by two cross sectional studies [45,46]. One out of 401 ADHD participants was diagnosed with CD, making the pooled prevalence of CD in ADHD 0.3% [45].

(a)

(b)
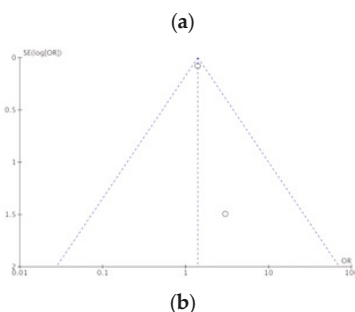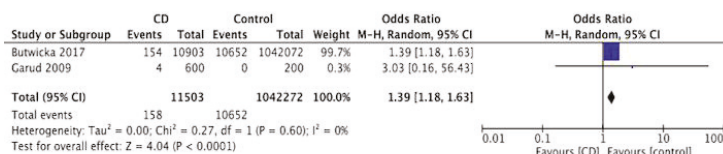
Figure 3. (**a**) Forest plot of pooled prevalence of ADHD in CD. (**b**) Funnel plot investigating distribution in ADHD studies.

3.1.1. GFD in ASD and ADHD

Two articles examined the role of the GFD in ASD, both observing no significant differences in behavioural symptoms between participants adhering to a GFD and those who did not [33,37]. Similarly, two articles examined the role of GFD in ADHD [40,41]. Both found significant improvements in behavioural symptoms, however, both studies are based on small sample sizes.

3.1.2. Limitations of Studies in ADHD and ASD

Firstly, several studies had small sample sizes [35–41,47]. This is especially important due to the heterogeneity of ASD, and therefore there is a particular need for large sample sizes [31]. However not all of the studies suffered from this limitation, Butwicka et al. (2017) and Ludvigsson et al. (2013) consisted of very large sample sizes, and therefore results held statistical power.

Secondly, not all of these studies controlled for patients being already on GFD, Ludvigsson et al. (2013) emphasises the importance of this, as it can cause levels of gluten related antibodies to fall resulting in false negative CD diagnosis. This is particularly relevant to ASD, because of high numbers of individuals with ASD adhering to a GFD [31]. Thirdly, despite much deliberation surrounding increases in GI symptoms and intestinal permeability in ASD mentioned in these articles, only two tested these theories [38,39]. Lastly, it is also worth considering that the majority of these studies for both ASD and ADHD concern pediatric populations, which is understandable as ASD and ADHD are both prevalent in childhood [31,34,35,37–42,45,46]. However, unlike ASD and ADHD, CD is not confined to childhood and is very prevalent later on in life [45].

3.2. Mood Disorders

This group of disorders contained the largest number of articles eligible for inclusion. Twenty articles in total, all investigating mood disorders in CD patients, accounted for a total of 16,412 participants [34,41,43,44,47–61]. Ten studies suggested a significant association between mood disorders and CD [34,47–55]. The majority of these studies were concerned with depression, with the second most common being anxiety.

3.2.1. Depression

Nineteen studies evaluated the presence of depression in CD patients. Out of a total of 16,300 participants, depression was found in 565. The prevalence of depression in CD was assessed by 11 case-controlled studies [44,51–58], 4 cross-sectional studies [43,47,59,60], 2 case series [52,61], and 2 cohort studies [49,50]. The pooled prevalence of depression in CD was 3.5%. The meta-analysis of the 11 case-controlled studies is summarized in a forest plot in Figure 4a, the odds of having depression was significantly higher in the CD groups compared to controls (OR 2.17, 95% CI 2.17–11.15, $p < 0.0001$). Figure 4b shows a funnel plot for these 11 studies.

(a)

(b)
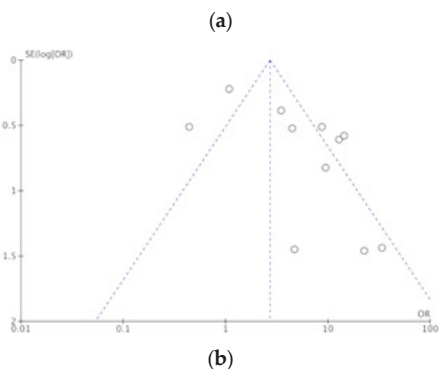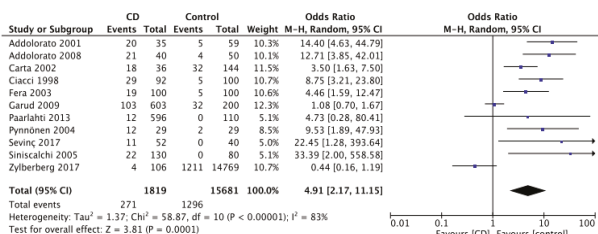
Figure 4. (**a**) Forest plot of pooled prevalence of depression in CD. (**b**) Funnel plot investigating distribution in depression studies.

3.2.2. Anxiety

Ten articles assessed anxiety in CD patients. Out of a total of 11,884 participants there were 443 cases of anxiety. The pooled prevalence was investigated by one cross sectional study [47] eight case-controlled studies [34,44,48,52–55,58] and one case series study [41]. The pooled prevalence of anxiety in CD was therefore 3.7%. A meta-analysis of seven of these case-controlled studies is summarized in a forest plot in Figure 5a, the odds of having anxiety was significantly higher in the CD groups compared to controls (OR 6.03, 95% CI 2.22–16.35, $p < 0.0001$). Figure 5b represents a funnel plot for these studies.

Study or Subgroup	CD Events	Total	Control Events	Total	Weight	Odds Ratio M–H, Random, 95% CI
Addolorato 2001	34	35	23	59	10.6%	53.22 [6.81, 415.98]
Addolorato 2008	28	40	8	50	16.1%	12.25 [4.44, 33.78]
Carta 2002	19	36	42	144	17.4%	2.71 [1.29, 5.73]
Fera 2003	51	100	5	100	16.2%	19.78 [7.41, 52.75]
Garud 2009	52	600	9	200	17.5%	2.01 [0.97, 4.16]
Pynnönen 2004	6	29	7	29	14.9%	0.82 [0.24, 2.83]
Sevinç 2017	10	52	0	40	7.4%	20.01 [1.14, 352.77]
Total (95% CI)		**892**		**622**	**100.0%**	**6.03 [2.22, 16.35]**
Total events	200		94			

Heterogeneity: Tau² = 1.35; Chi² = 33.52, df = 6 (P < 0.00001); I² = 82%
Test for overall effect: Z = 3.53 (P = 0.0004)

(**a**)

(**b**)

Figure 5. (**a**) Forest plot of pooled prevalence of anxiety in CD. (**b**) Funnel plot investigating distribution in anxiety studies.

3.2.3. Bipolar Disorder

Out of these articles, eight provided data concerning the prevalence of bipolar disorder in CD, this was made up of one cross sectional study [59] two case series studies [41,62] one cohort study [50], and four case-controlled studies [43,48,52,53]. These studies add up to 14,820 participants, 33 of these were found to already have, or meet the criteria, for a bipolar disorder diagnosis. Producing a pooled prevalence of bipolar disorder in CD of 0.2%. The meta-analysis of the four case-controlled studies is summarized in a forest plot in Figure 6a, no statistically significant differences were detected for bipolar disorder and CD, compared to controls (OR 2.35, 95% CI 2.29–19.21, $p = 0.43$). Figure 6b presents a funnel plot for these studies.
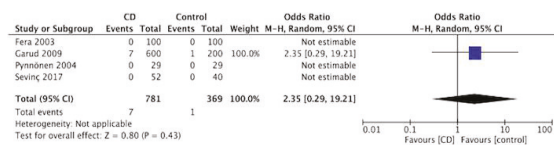
(a)
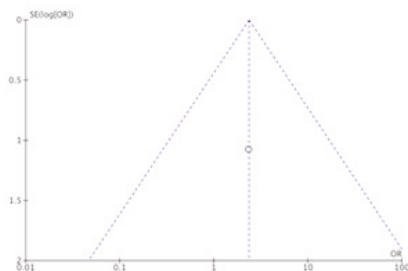
(b)

Figure 6. (**a**) Forest plot of pooled prevalence of bipolar disorder in CD. (**b**) Funnel plot investigating distribution in bipolar disorder studies.

3.2.4. GFD in Mood Disorders

Nine studies provided data concerning the role of the GFD, four of which reported no association between anxiety and depression with adherence to a GFD [48,53,56,58]. Some claimed that adhering to a GFD causes worsening or persistence of depressive symptoms [51,55,57]. Furthermore, Addolorato (2001) reported improved anxiety but sustained depression symptoms in patients on a GFD.

3.2.5. Limitations in Studies of Mood Disorders

Firstly, self-reported measures of both anxiety and depression, as well as GFD adherence limit validity [59,60]. Additionally, much conflict exists in literature surrounding the duration of GFD. It has been argued that any effect of GFD should be investigated longitudinally after at least one year of a GFD, as otherwise results lack reliability [41,55]. This has not been the case for the majority of these studies. Also, several potential confounding factors are highlighted, that are not always controlled for, such as autoimmune thyroiditis, family history of mental illness, and severity of CD symptoms [52,60]. Additionally, cultural differences regarding the social burden of the GFD should also be taken into consideration. For example, in Italy this is likely to be more prominent, as food holds more cultural and social importance when compared to other countries [55,58].

3.3. Schizophrenia and Other Psychotic Disorders

A total of six articles investigated the prevalence of schizophrenia and other psychotic disorders in CD, adding up to a total of 11,741 participants. This assessment of the prevalence of schizophrenia and other psychotic disorders in CD was performed by one cross sectional study [43] four case-controlled studies [34,44,48,52] and one case series study [41]. Out of these a total of 12 were identified with either schizophrenia or another psychotic disorder, producing a pooled prevalence of schizophrenia and other psychotic disorders in CD of 0.1%. Ten of these came from Butwicka et al. (2017) who had a sample of 10,903 with CD, and then one each from Garud et al. (2009) and Vaknin et al. (2004). None of these studies specifically concluded that there was a significant association between schizophrenia or other psychotic disorders and CD. The meta-analysis of three of the case-controlled studies is summarized in a forest plot in Figure 7a, no statistically significant differences were detected with schizophrenia

or other psychotic disorders and CD compared to controls (OR 0.46, 95% CI 0.02–10.18, *p* = 0.62). Figure 7b) displays a funnel plot for these studies.

(a)

(b)
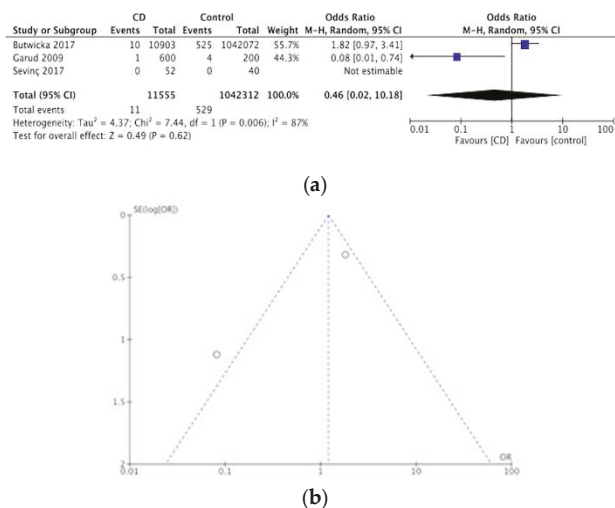
Figure 7. (**a**) Forest plot of pooled prevalence of schizophrenia and other psychotic disorders in CD. (**b**) Funnel plot investigating distribution in schizophrenia and other psychotic disorders studies.

3.3.1. GFD in Schizophrenia and Other Psychotic Disorders

None of the studies examined the impact of the GFD in cases of schizophrenia and other psychotic disorders [41,48]. Therefore, there is very limited information on the subject.

3.3.2. Limitations in Studies on Schizophrenia and Other Psychotic Disorders

This was the category with the least number of eligible articles, resulting in restricted information to interpret, which is a criticism in itself. Another limitation is that several studies were investigating cases of schizophrenia or other psychotic disorders in CD patients from pediatric populations [34,41,48,52]. This may be a significant limitation, as it is currently understood that the onset of schizophrenia or other psychotic disorders during childhood is uncommon and is instead most likely to present between adolescence and early adulthood.

3.4. Eating Disorders

Out of nine articles concerning eating disorders and CD, four concluded that there is a significant association. The prevalence of eating disorders within CD was investigated by one cohort and case control study [63] four case control studies [34,44,48,52] and three cross sectional studies [43,64,65]. Out of 29,977 CD patients, coexisting eating disorders were detected in 221, creating a pooled prevalence of eating disorders in CD of 0.7%. The meta-analysis of the three case-controlled studies is summarized in a forest plot in Figure 8a, the odds of having an eating disorder was significantly higher in the CD groups compared to controls (OR 1.62, 95% CI 1.37–1.91, *p* < 0.00001). Figure 8b shows a funnel plot for these studies.

(**a**)

(**b**)
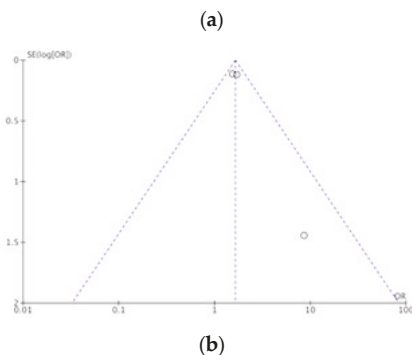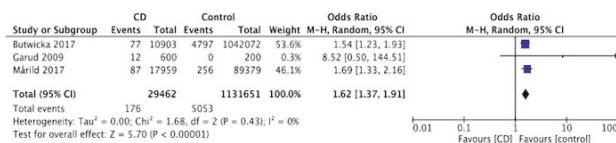
Figure 8. (**a**) Forest plot of pooled prevalence of eating disorders in CD. (**b**) Funnel plot investigating distribution in eating disorder studies.

The prevalence of CD within eating disorders was assessed by one cross sectional study [65] and one case-controlled study [66,67]. Amongst 841 patients with eating disorders, 15 cases of CD were determined, therefore the pooled prevalence of CD in eating disorders was 1.8%.

3.4.1. GFD in Eating Disorders

Only one study that explored associations between eating disorders and CD also investigated the impact of the GFD [66]. This study obtained a sample where all participants had a diagnosis of anorexia nervosa, one participant was found to also have CD. No differences in disordered eating were found whilst following a GFD, however resolution of amenorrhea was noted in this individual [66].

3.4.2. Limitations in Studies on Eating Disorders

Only two out of these nine articles had a sufficiently large sample size [34,63]. As a result, the reliability of results in studies with smaller samples risk being compromised. Another limiting factor is gender, as male participants were often excluded [63,64,67]. Even in those featuring male participants, male sample sizes were always very small. [64,66,67]. Welch, Ghaderi, and Swenne (2015) acknowledge this in their study. Lastly, screening for CD requires ingestion of sufficient amounts of gluten in order to avoid false negative results. This risk is much higher in participants with eating disorders [66].

4. Discussion

This systematic review has identified a significant increased risk for ASD, ADHD, depression, anxiety, and eating disorders amongst patients with CD compared to healthy controls. No significant risk was identified for bipolar disorder or schizophrenia.

Clearly such findings are relevant to clinical practice, as both ASD and ADHD patients are often advised to adopt a GFD to reduce behavioural problems [38,40–46]. There is no rational for doing so unless the patient has been tested for CD prior to adopting a GFD. There is an urgent need for studies investigating the effects of a GFD in these populations as what has been published so far has not been adequately powered, the duration of the intervention was suboptimal and the monitoring of the strictness of adherence to a GFD using repeat serological testing was not undertaken [33,37].

Associations between CD and neurodevelopmental disorders could suggest an unknown biological cause with some invoking the gut–brain axis relationship [34,37]. However, such biological explanations lack evidence, therefore further research is required [33,39]. Of interest is the role of the cerebellum in ASD and ADHD. The cerebellum has emerged as one of the key brain regions affected in non-motor disorders, including autism spectrum disorder and attention deficit-hyperactivity disorder. The cerebellum is the principle brain target in both CD and gluten sensitivity.

Examining the prevalence of depression and anxiety demonstrated significant increased risk in CD patients compared to controls. This is in keeping with anecdotal reports from health professionals that care for patients with CD, that both anxiety and depression are prominent features in this group. No statistically significant differences were identified for bipolar disorder in CD patients. Research often distinguishes between pre and post CD diagnosis to draw hypotheses concerning anxiety and depression in CD, claiming adherence to a GFD causes anxiety to subside whilst depression often persists [34,55]. Social implications of the GFD (social isolation, avoiding going out because of the risk of contamination, having to always declare the condition amongst friends and colleagues, having to explain the diagnosis of CD as opposed to a life choice of GFD, etc.) are blamed for this [34,47,50,51,55,56,59]. Psychological support beyond simply advising a GFD is argued in several studies, may promote acceptance and subsequent adherence to the GFD, as well as reducing the risk of anxiety and depression [47,53,57].

Our meta-analysis assessing the prevalence of schizophrenia and other psychotic disorders in CD patients, found no significant difference compared to healthy controls. However, a portion of the wider literature still argues for an association [68,69]. There are several case reports reporting patients with acute psychosis developing at the same time as a diagnosis of CD being made. The argument in favour of a link is based on the fact that these patients seem to improve on a strict GFD. The identification of immune mediated psychosis in the context of NMDA encephalitis for example, also provides some evidence for autoimmunity having a role in these disorders.

Significantly increased prevalence of eating disorders in CD patients was detected in this meta-analysis. Theories often relate back to the vigilance required for a GFD, as this may produce a fixation with food intake as a whole [63,64]. The prominent gastrointestinal symptoms that can be seen in the context of CD may also play a part in driving the fear of eating. The risk of misdiagnoses due to similarities between eating disorders and CD symptoms is often discussed in literature, for example GI and malnutrition symptoms are present in both [63,66]. For this reason, monitoring and awareness of the possibility of CD is described as crucial [63,64,66,67].

Associations between depression, anxiety, and eating disorders are apparent as a result of the psychological and social implications of CD, however specific biological causes for these disorders are uncertain. Psychological and social implications are less clear for ASD, ADHD, where biological causes are speculated to play a more prominent role. Further research is required to add clarity to what seems to be a rather conflicting literature.

5. Limitations

There was a significant heterogeneity between studies included in this review which is reflected in the funnel plots. This could be explained by the existence of grey literature or simply might reflect the fact that the subject is still understudied and that more studies should be carried out in the future.

Secondly, a single database was utilized to conduct the literature search for this study. This may have caused some studies to be excluded. However, we have checked the reference lists of every included study to identify additional seminal publications.

Finally, the role of GFD has been studied but only in observational studies, the majority of which were conducted in small populations. By definition, observational studies provide low evidence and therefore no recommendations can be made based on this review [70]. However, RCTs on the matter might shed further light into the matter.

6. Conclusions

The findings for this systematic review and meta-analysis provide support for the notion that CD has an increased risk for specific psychiatric disorders probably through indirect adverse effects on mental health and social life. However further research is required to investigate the pathophysiology of such associations.

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Review

The Neuropathology of Gluten-Related Neurological Disorders: A Systematic Review

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Abstract: Gluten-related neurological disorders (GRND) represent a spectrum of neurological manifestations that are triggered by gluten. In coeliac disease, a T-cell mediated enteropathy is triggered by gluten in genetically predisposed individuals. The underlying pathological mechanism of the neurological dysfunction is not yet clear. The aim of this review is to collate existing neuropathological findings in GRND as a means of aiding the understanding of the pathophysiology. A systematic search of the Pubmed Database yielded 188 articles, of which 32 were included, containing 98 eligible cases with a description of pathological findings in GRND. In gluten ataxia, loss of Purkinje cells, atrophy, gliosis and astrocytosis were apparent, as well as diffuse lymphocytic infiltration and perivascular cuffing with lymphocytes. In patients with large-fiber neuropathy, nerve biopsies revealed axonopathy, loss of myelinated fibers and focal and perivascular infiltration by inflammatory cells. Inflammatory infiltrate was also observed in muscle in myopathy and in cerebrum of patients with encephalopathy and patients with epilepsy. Such changes were not seen in skin biopsies from patients with small fiber neuropathies. The findings from this systematic review suggest an immune mediated pathogenesis for GRND. Future research should focus on the characterization of the inflammatory cell infiltrates and identifying target epitopes.

Keywords: gluten; coeliac disease; neurological disorders; gliadin; ataxia; neuropathy; myopathy; encephalopathy

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1. Introduction

Coeliac disease (CD) is an autoimmune disorder triggered by the ingestion of gluten in genetically susceptible individuals. Classical CD typically presents to gastroenterologists with a combination of abdominal pain, diarrhea, bloating, anemia and other gastrointestinal symptoms [1]. Over the past decades, a shift in presentation has been observed from these classic symptoms to extraintestinal symptoms in children as well as adults [2,3]. A wide range of extraintestinal manifestations has been attributed to CD, changing the classic perception of a disease limited to the intestine, to a multisystem disorder. CD can therefore manifest with dental problems, consequences of malabsorption, skin and neurological disorders [4]. Some patients can develop refractory CD. This is characterized by persistent

villous atrophy despite strict adherence to gluten-free diet. Symptoms are often severe with diarrhea, weight loss and severe malabsorption [5].

Cerebellar ataxia and peripheral neuropathy are the most common neurological manifestations [5,6]. These can also occur in the absence of enteropathy and are sometimes referred to as non-coeliac gluten sensitivity, or simply gluten sensitivity [7,8]. Not much is known about the pathogenesis of such neurological manifestations. However, both humoral and cell-mediated immune mechanisms have been proposed. The aim of this systematic review is to analyze the published neuropathology of confirmed cases of gluten-related neurological dysfunction in an attempt to aid our understanding of the pathogenesis.

2. Materials and Methods

2.1. Literature and Search Strategy

A systematic search was conducted on May 1 2019, using the Pubmed database. The following terms were applied, searching title and abstract (TiAb): celiac OR coeliac AND neuropathy OR polyneuropathy OR ganglionopathy OR neuronopathy OR ataxia OR migraine OR headache OR myopathy OR myositis OR epilepsy OR "movement disorders" OR encephalitis OR encephalopathy AND pathology OR pathological OR neuropathology OR neuropathological or post-mortem. Complementary to this electronical search, we scanned the reference lists of all included articles to increase the yield of eligible papers.

Inclusion and exclusion criteria

Papers were considered for inclusion if they met the following criteria:

1. Original clinical studies
2. Human subject study
3. Pathological studies in patients with coeliac disease or gluten sensitivity, suffering from neurological illness

Papers were excluded in case they met the following exclusion criteria:

1. If there was no histopathological description of neuronal/brain tissue or muscle
2. No evidence of coeliac disease or gluten sensitivity
3. Reviews, book chapters, editorials
4. Duplicate articles

All abstracts were screened for eligibility. Full articles were read if the abstract was not sufficient in scoring all inclusion and exclusion criteria. In cases where inclusion criteria were questioned, the paper was discussed with at least two authors (PZ & MH) to reach consensus. Careful consideration of the inclusion and exclusion criteria regarding the article in question, led to unanimous decisions of inclusion or exclusion by MR, PZ and MH.

2.2. Ethical Considerations

No ethical approval was needed as this was a systematic review.

3. Results

3.1. Study Characteristics

The search strategy identified 188 articles. After detailed assessment, 24 papers met the inclusion criteria. Eight additional articles were identified by scanning reference lists of the included papers. In total, 32 articles were included in this review (Figure 1).

All studies described in the articles were categorized by neurological disorder. Study characteristics and main neuropathological findings are summarized in Table 1.

A: celiac OR coeliac OR gluten

B:): Neuropathy OR polyneuropathy OR ganglionopathy OR neuronopathy OR ataxia OR migraine OR headache OR myopathy OR myositis OR epilepsy OR "movement disorders" OR "movement disorders" OR encephalitis OR encephalopathy AND pathology OR pathological OR neuropathology OR neuropathological or post-mortem

188 articles identified

Excluded 164 articles:

1. No histopathological description of neuronal tissue
2. No evidence of coeliac disease or gluten sensitivity
3. Reviews, book chapters, editorials
4. Duplicate articles

24 articles met inclusion criteria

Scanning reference lists
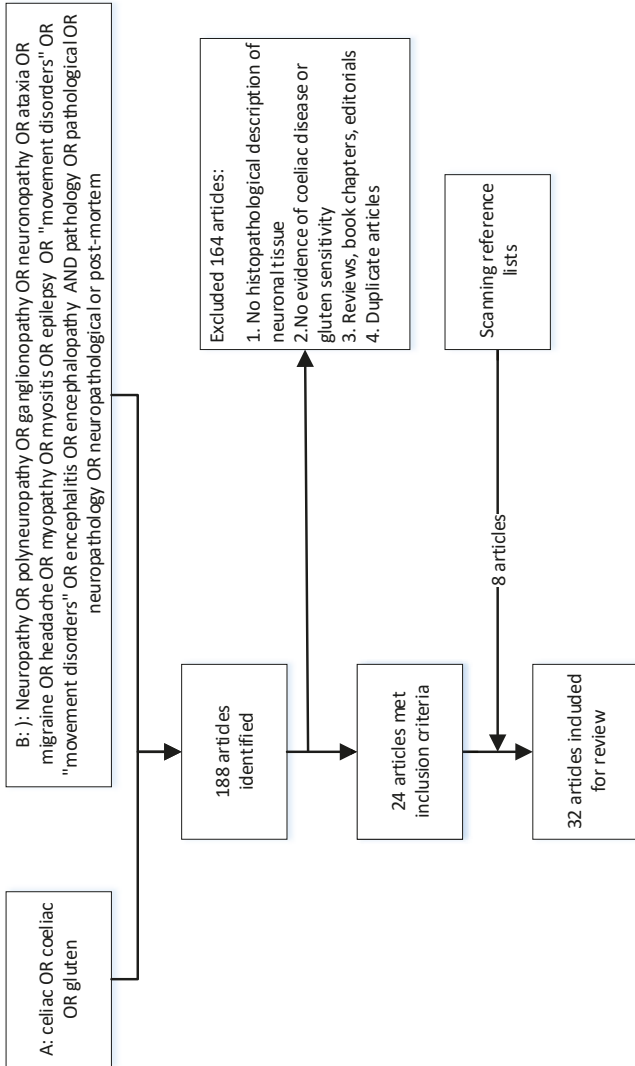
8 articles

32 articles included for review

Figure 1. Prisma chart illustrating the literature inclusion/exclusion flow.

Table 1. Study characteristics and main neuropathological findings.

Neurological Disorder Papers n First Author/Year/Country	Patients n	Tissue	Main Neuropathological Findings
Ataxia 9			
Bhatia 1995, UK	18	Brain, Spinal cord	Loss of Purkinje cells and cells in inferior olives; Cerebellar gliosis; Astrocytosis of the granular layer, dentate nucleus & inferior olives
Cooke 1966, UK (I) *	9	Brain, Spinal cord	Loss of Purkinje cells; atrophy and gliosis of the dentate nucleus, cerebrum, inferior olives, thalamus and hypothalamus; demyelination of post. & ant.-lat. columns; focal perivascular lymphocytic cuffing, chromatolysis and sudanophil lipophages throughout the CNS.
Finelli 1980, USA	1	Brain, Spinal cord	Loss of Purkinje and granular layer cells; Neuronal loss & gliosis basal ganglia, inferior olives, substantia nigra; Demyelination ant. & lat. corticospinal tracts
Hadjivassiliou 1998, UK	2	Brain, Spinal cord	Loss of Purkinje cells; Cerebellar atrophy & astrocytic gliosis & vacuolation of neutrophils; Diffuse infiltration of lymphocytes & perivascular cuffing of T-lymphocytes in the cerebellum and the post. columns
Hadjivassiliou 2006, UK **	1	Brain	Capillary changes in the white matter, hippocampus and olives marked by vascular and perivascular inflammatory cell infiltrates (CD68+ cells and a smaller CD45Ro+ cell population). Purkinje cell loss and Bergmann gliosis and loss of neurons in the inferior olives
Kinney 1982, USA	1	Brain	Loss of Purkinje cells; atrophy, gliosis of the dentate nucleus, cerebellar granular layer, thalamus, hypothalamus & periaqueductal grey; Senile plaques in the neocortex & hippocampi; Cerebral gliosis subcortical & white matter
Mittelbronn 2010, Germany	1	Brain	Loss of Purkinje cells & cerebellar granular layer cells; Cerebellar atrophy and astrocytic gliosis; Severe neuronal loss inferior olives & accumulation of corpora amylacea. Cerebral reactive astrogliosis and microglial activation
Nanri 2011, Japan	1	Brain	Inflammation dominated by CD8+/granzyme B+ & CD20/CD138- diffuse infiltrates & perivascular cuffing in the cerebellum and brainstem. Loss of Purkinje cells; Minimal cerebellar atrophy; Mild Bergmann gliosis. Empty basket cells, Edematous splitting of Purkinje cell layer, loss of granular cells. No lymphocytic infiltration (CD3-, CD4-, CD8-, CD20-, CD68-, CD79A-)
Tuzun 2001, Turkey	1	Skin	skin biopsy: Periodic Acid Schiff-positive inclusions, diastase resistant intracellular inclusion bodies in apocrine sweat gland cells
Neuropathy 10			
Bramagan 2005, USA	37	Skin	Reduced epidermal nerve density, distal > proximal
Cooke 1966, UK (I) *	8	Skin	Axonal swelling, loss of myelinated fibers, focal proliferation of sarcolemmal nuclei and collateral reinnervation
Cooke 1966, UK (II)	11	Nerve	
Chin 2003, USA	3	Sural nerve	Mild to moderately severe chronic axonopathy with loss of myelinated fibers
De Sousa 2006, USA	6	Skin	Morphological changes & a reduced epidermal nerve density
Hadjivassiliou 2006, UK **	3	Sural nerve	Focal inflammatory cell infiltrate in the epineurium & perivascular cuffing of lymphocytes; Patchy loss of myelinated fibers & occasional degeneration

Table 1. *Cont.*

Neurological Disorder / Papers *n* / First Author/Year/Country	Patients *n*	Tissue	Main Neuropathological Findings
Hadjivassiliou 2010, UK	2	Spinal cord	Degeneration of the dorsal columns; Preservation of the ant.-lat. white matter; Subtotal loss of myelin; Axonal loss; Lymphocytic infiltration
Simonati 1998, Italy	1	Sural nerve	Chronic axonal neuropathy; Significant loss of myelinated fibers & Schwann cell nuclei; Low density of unmyelinated fibers; No inflammatory cells objectified
Souayah 2008, USA	2	Skin	Low to normal epidermal nerve fiber density; Sparse nerve fibers; axonal swelling; increased branching; uneven distribution of epidermal fibers in calf and thigh both
Squintani 2009, Italy	1	Sural nerve	Loss of myelinated axons; Axonal degeneration with focal distribution in different fascicles; Mild perivascular mononuclear cell infiltration of epineural blood vessels; Thickened perineurium
Myopathy 7			
Alawneh 2008, Jordan	1	Muscle	Muscle necrosis; Neutrophilic infiltration; Secondary leukocytoclastic vasculitis
Danielsson 2017, Sweden	13	Muscle	Inflammatory infiltrates & muscle fiber degeneration
Hadjivassiliou 1997, UK	2	Muscle	Inflammatory myopathy & Basophilic rimmed vacuoles
Hadjivassiliou 2007, UK	13	Muscle	Internal nuclei; Basophilic rimmed vacuoles; fiber splitting; Endomysial chronic inflammatory cell infiltrate (CD3+ cells), Fibrosis
Hendriksson 1982, Sweden	5	Muscle	Basophilic sarcoplasm; Vesicular nuclei, Muscle Fibre Atrophy, Splitting & Internally placed nuclei
Kleopa 2004, Cyprus	1	Muscle	Inflammatory cell infiltrates; Rimmed vacuoles; Sural nerve biopsy: Chronic active axonopathy; Loss of myelinated fibers; Regeneration & Necrotic fibers
Williams 2003, USA	1	Muscle	Basophilic rimmed vacuoles
Encephalopathy 5	9		
Brucke 1988, Austria	1	Brain	Brain edema; Periventricular lesions; Inflammatory necrosis; Demyelinated fibers; hypertrophy of the inferior olives; Vermal Bergmann gliosis; Lymphocyte infiltration of the pons and mesencephalon
Dimberg 2007, USA	1	Brain	Loss of Purkinje cells & granular layer cells; Astrocytic gliosis of the frontal, parietal, occipital cortices, globus pallidus, hippocampus, midbrain, pons, medulla; Widespread perivascular lymphocytosis in the cortex hippocampus and temporal gyrus, frontoparietal atrophy;
Hu 2006, USA	5	Brain	Non-specific gliosis & astrocytosis; Ubiquitin-positive inclusions in 1 patient
Keller 2006, USA	1	Brain / Spinal cord	Loss of Purkinje cells; Neuronal loss of the dentate nucleus; Perivascular cuffing of lymphocytes; Arterial deformations of the pons, midbrain, thalamus and basal ganglia; Mild degeneration of the pyramidal tract & posterior columns; Loss of myelinated nerve fibers in the nerve roots
La Mantia, 1998, Italy	1	Brain	Brain edema; Calcifications, Increased vascularity, Mild neuronal loss, Reactive gliosis & Demyelination
Epilepsy 2	2		
Bye 1993, Australia	1	Brain	Pial angiomatosis consisting of groups of small veins entrapped by collagen; Severe sclerosis of veins; Lymphocytic perivascular cuffing in the cortical neuropil. Atrophy of the white matter and influx of macrophages & reactive gliosis (all occipital lobe)
Orstavik 1997, Norway	1	Brain	Megalencephaly

Post: posterior, lat: lateral, ant: anterior, CNS: central nervous system, * & **: Same article.

3.2. Ataxia and Gluten

Ataxia refers to loss of coordination, clumsiness and gait instability, and has multiple possible underlying etiologies. Ataxia has been described concomitant with CD and GS [9]. Cooke and Smith where the first to report a case series of 16 patients with established coeliac disease and concomitant ataxia and/or neuropathy. Post-mortem findings were reported in nine cases. Eight patients were male. The diagnosis of CD, based on jejunal biopsy, preceded neurological symptoms by more than two years in seven cases. Five patients had classical symptoms (e.g., weight loss and diarrhea) at the time of neurological presentation. All patients presented with steatorrhea, two patients with anemia, two were diagnosed with osteomalacia and one had a vitamin B12 deficiency. Primary neurological complaints were progressive gait instability, weakness, numbness and pain of the limbs. Adherence to a gluten-free diet did not seem to influence the neurological symptoms. The diagnosis of CD and initiation of a gluten-free diet preceded the diagnosis of ataxia by decades in seven patients. Moreover, in patients with simultaneous diagnosis of ataxia and CD, neurological symptoms slowly progressed despite the initiation of a gluten-free diet. However, no data on strictness of GFD or serology were available in this report. The median age at time of death was 51 years. In six out of nine cases, cerebellar neuronal atrophy and loss of Purkinje cells was demonstrated, in five cases accompanied by gliosis, especially in the dentate nucleus [8]. The loss of Purkinje cells in a patient with gluten ataxia is illustrated in Figure 2. Pathological findings of the cerebrum were neuronal atrophy, gliosis and chromatolysis of the cortex. The inferior olives, the thalamus and hypothalamus showed gliosis and atrophy as well. Demyelination of the posterior columns and anterolateral columns of the spinal cord was demonstrated in eight cases. The spinocerebellar tracts were heavily affected in three patients. Perivascular lymphocytic cuffing, chromatolysis and sudanophil lipophages were demonstrated in various areas of the central nervous system.
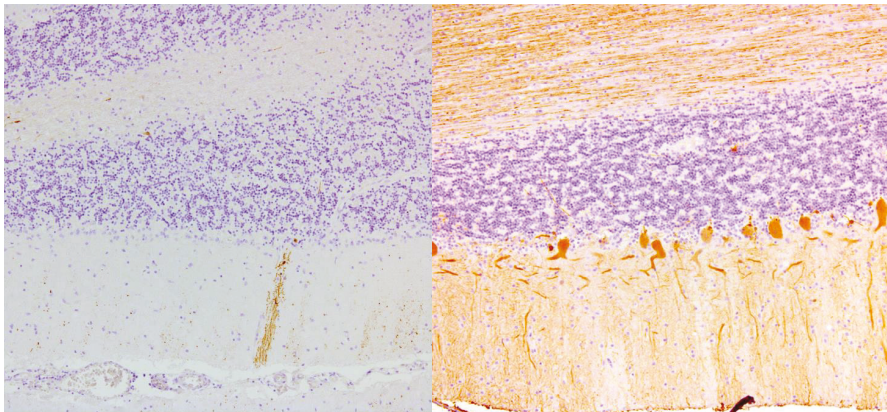
Figure 2. Complete loss of Purkinje cells in the cerebellar cortex of a gluten ataxia patient (left). Normal cerebellum from a control patient on the right shows the single layer of Purkinje cells (calbindin and hematoxylin staining, magnification 100×).

In another case report a 27-year-old female with a slowly progressive ataxia, dysarthria and myoclonic jerks of the limbs was described [10]. Further investigation revealed elevated anti-gliadin antibodies and subtotal villous atrophy with inflammatory infiltrate in the lamina propria.

Bhatia and colleagues reported four cases of progressive myoclonic ataxia developing after the diagnosis of CD [11]. Autopsy was performed in one patient (male, 68 years old at time of death). He was diagnosed with dermatitis herpetiformis in his twenties and had been successfully treated with medication. Neurological complaints were first apparent at the age of 41 years, with progressive involuntary jerking of the limbs, gait ataxia and cognitive dysfunction. Despite strict adherence to

a gluten-free diet, villous atrophy persisted and neurological symptoms progressed. No serological evidence of strict gluten-free diet was available. At 68 years of age, the patient committed suicide. Autopsy revealed cerebellar atrophy and histological examination demonstrated loss of Purkinje cells, Bergmann gliosis, astrocytosis mainly in the granular layer and dentate nucleus. Neuronal loss and astrocytosis was seen in the inferior olives as well.

Finelli [12] and Kinney [13] reported similar cases with CD and myoclonic ataxia. Both patients were male and had a progressive cerebellar syndrome starting in their fifth and sixth decades, respectively. Histological examination demonstrated severe loss of Purkinje cells and granule cells, gliosis of the dentate nucleus and inferior peduncles. In the spinal cord, demyelination of the anterior and lateral corticospinal tracts, as well as of the posterior columns and lateral corticospinal tracts was also observed. In a series of patients with CD and ataxia published by Hadjivassiliou and colleagues, two autopsies were reported [14]. The findings in one of the patients were dominated by Purkinje cell loss, astrocytic gliosis, vacuolation of neutrophils, diffuse infiltration of lymphocytes and perivascular cuffing by T-lymphocytes in white matter of the cerebellum. Infiltration of lymphocytes was also demonstrated in the posterior columns of the spinal cord. No abnormalities were found in the cerebellum nor in the cerebrum of the second patient. However, there was evidence of spinal cord degeneration of the posterior columns suggesting that the etiology of the ataxia was due to posterior column sensory loss (sensory ataxia).

Mittelbronn et al. were the first to attempt to characterize the nature of the lymphocytes infiltrating the CNS of a patient with gluten ataxia [15]. In addition to the reported loss of Purkinje cells, granular cells and loss of neurons with accumulation of corpora amylacea in the inferior olives, widespread diffuse infiltration and perivascular cuffing of lymphocytes was reported. Inflammation was characterized by the presence of CD8+ and granzyme B+ lymphocytes and by microglial cell activation. No CD20+ or CD138+ cells were observed. However, in a case described by Nanri with cerebellar atrophy, Purkinje cell loss and mild Bergmann gliosis, no lymphocytic infiltration was observed (CD3-, CD4-, CD8-, CD20-, CD68-, CD79A-) [16]. In a case of a 32-year-old female that died from a rapidly progressive ataxia and neuropathy, CD was diagnosed at the time of neurological presentation [17]. During autopsy capillary changes were found in the white matter, hippocampus and olives marked by vascular and perivascular inflammatory cell infiltrates (CD68+ cells and a smaller CD45Ro+ cell population). Purkinje cell loss and Bergmann gliosis were marked in the cerebellum and loss of neurons was observed in the inferior olives. Further examination of the spinal roots revealed inflammatory cell infiltrates of lymphocytes and macrophages.

3.3. Large Fibre Neuropathy and Gluten Sensitivity

Peripheral neuropathy results from damage of the peripheral nervous system. The underlying pathological mechanism can be driven by hereditary factors, drug-induced damage, infection, inflammation and metabolic conditions such as diabetes and vitamin deficiencies. The cause of neuropathy in the context of CD and GS has frequently been attributed to malabsorption and inflammation.

Cooke and Smith reported 11 cases with CD and peripheral neuropathy [9]. All but two patients were diagnosed with CD years prior to their neurological diagnosis. The median age at neurological presentation was 49 years and 64% were male. The patients complained of numbness, tingling and pain of the lower extremities and gait instability. Three patients lost their ability to write. In the workup of their neuropathy, low serum vitamin B12 was found in three cases. Osteomalacia was demonstrated in five cases, and three patients tested positive for toxoplasmosis. Muscle biopsies (flexor digitorum sublimis, palmaris longus and flexor carpi radialis) were performed. Tissue containing terminal nerve bundles were then further processed and studied using electron microscopy [18]. Common findings were axonal swelling, loss of myelinated fibers, focal proliferation of sarcolemmal nuclei and collateral re-innervation.

Chin et al. described three patients that presented with distal paresthesia and dysesthesia [19]. Diagnosis of CD was previously established in two cases and simultaneously diagnosed with the neuropathy in the third. Only one patient adhered to a strict gluten-free diet at the time of presentation. All patients underwent sural nerve biopsies, demonstrating mild to moderately severe chronic axonopathy with loss of myelinated fibers.

Squintani reported a case of polyneuropathy in a 49-year-old male that presented with painful paresthesia and progressive generalized muscle atrophy, leading to gait instability [20]. Blood tests showed hepatitis B infection. Consequently, his neurological complaints were attributed to a secondary vasculitis. After two years of disease progression, additional tests revealed high antibody levels of anti-gliadin and anti-transglutaminase 2 (TG2). The patient was also positive for endomysium antibodies. The diagnosis of CD was confirmed with a duodenal biopsy. A sural nerve biopsy demonstrated loss of myelinated axons and axonal degeneration. Mild perivascular mononuclear cell infiltration of epineural blood vessels but without fibrinoid necrosis was seen. Nerve fascicles showed degeneration and the perineurium was abnormally thick suggestive of ischemic damage. A year after commencing a gluten-free diet, and six months after steroid treatment, the symptoms improved dramatically.

Simonati reported the findings of a nerve biopsy performed in a three-year-old child diagnosed with CD that presented with a progressive polyneuropathy [21]. Blood tests showed no evidence of malabsorption resulting in vitamin deficiencies or metabolic disorders. A sural nerve biopsy was performed, demonstrating chronic axonal neuropathy, significant loss of myelinated fibers and Schwann cell nuclei. The density of unmyelinated fibers was low, and no inflammatory cells were identified.

Hadjivassiliou et al. reported on 215 patients with axonal neuropathy [17]. After thorough examination, 140 patients were left with the diagnosis of chronic idiopathic axonal polyneuropathy, 47 (34%) of these tested positive for IgA or IgG anti-gliadin antibodies. Two patients had a sural nerve biopsy. In the first patient a focal inflammatory cell infiltrate in the epineurium was observed, as well as perivascular cuffing of lymphocytes and patchy loss of myelinated fibers and occasional degeneration. In the second patient, no evidence of cellular inflammation was found.

3.4. Small-Fibre Neuropathy and Gluten Sensitivity

Souayah reported two cases of confirmed small-fiber neuropathy in patients with CD [22]. The first patient was diagnosed two years following the initial neurological complaints of progressive numbness, tingling and electric-like pains. Skin biopsies of the calf and the thigh were performed. Histopathological examination of the skin biopsies revealed a low to normal epidermal nerve fiber density. Mild qualitative changes were seen in the thigh. Sparse nerve fibers, axonal swelling, increased branching, uneven distribution of epidermal fibers in calf and thigh resembling small-fiber neuropathy were seen. Biopsy results of the second patient showed similar findings. This patient had persistent diarrhea despite adherence to a gluten-free diet for over 17 years, suggestive of the possibility of refractory coeliac disease (RCD).

De Sousa and colleagues characterized 62 patients with sensory neuropathy [23]. In 50% of the cases the small-fiber neuropathy was considered of unknown cause. Eleven of these patients had high CD-related antibody titers. Six patients had biopsy-proven CD. Two of them had morphological changes on skin biopsy. Four also had a reduced epidermal nerve density below the 5th percentile of normality.

Brannagan et al. reported six women and two men with biopsy-proven CD and small-fiber neuropathy [24]. In seven cases neurological symptoms preceded the diagnosis of CD. The epidermal nerve density was measured in number of fibers per millimeter. Distal leg and thigh skin biopsies showed that the density was lower in the distal leg biopsies in five out of eight cases.

In another study by Hadjivassiliou et al., seven patients with neuropathy and sensory ganglionopathy were reported [25]. Autopsy was performed on three patients, of which one was previously reported. The other two cases were characterized by spinal cord abnormalities

with degeneration of the dorsal columns (Figure 3), and a subtotal loss of myelin, axonal loss and lymphocytic infiltration.
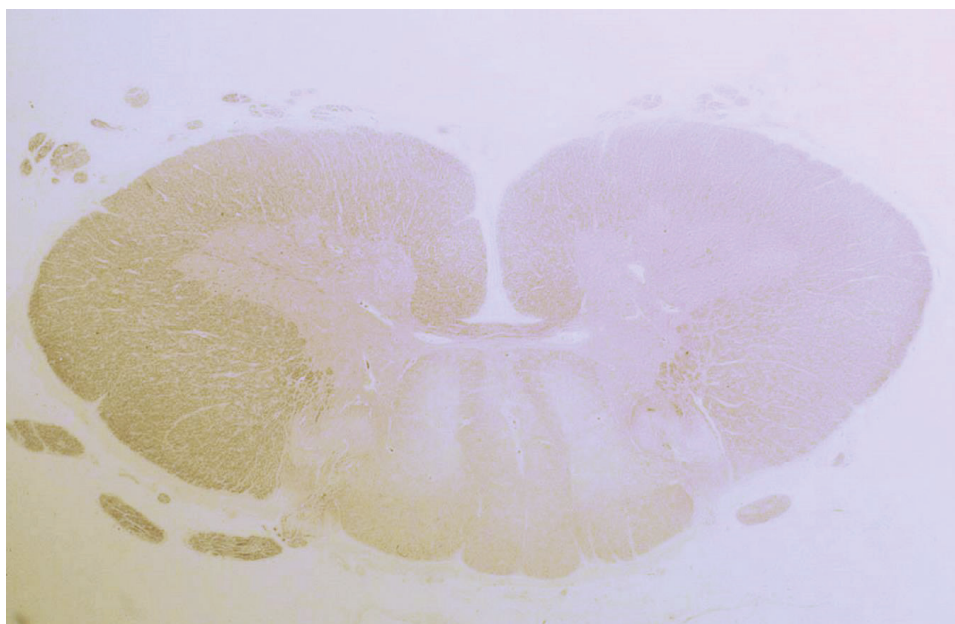
Figure 3. Degeneration (loss of tissue and a pale appearance) of the posterior column of the spinal cord in a patient with gluten ataxia and sensory ganglionopathy.

3.5. Myopathy and Gluten Sensitivity

Myopathy refers to an abnormality in structure and metabolism of skeletal muscle cells, leading to weakness and often wasting. A CD prevalence of 4.5% in a cohort of patients with idiopathic inflammatory myopathy was reported in one study [26].

Hadjivassiliou et al. described two cases (a 49-year-old female and a 64-year-old male) presenting with progressive pain, weakness, unsteady gait and diarrhea [27]. Muscle biopsies demonstrated an inflammatory myopathy with basophilic rimmed vacuoles suggestive of inclusion body myositis.

Williams et al. reported a similar case of a 51-year-old woman with concurrent CD and idiopathic thrombocytopenic purpura [28]. Muscle biopsy findings were consistent with inclusion body myositis. Henriksson and colleagues reported five patients with polymyositis and adult CD [29]. Muscle biopsies revealed inflammatory cell infiltrate (mostly lymphocytes), muscle fiber necrosis and myophagia.

One patient described by Kleopa et al. had concomitant vitamin E deficiency and an elevated alkaline phosphate [30]. Muscle biopsy revealed inflammatory cell infiltrates and rimmed vacuoles. A sural nerve biopsy showed chronic active axonopathy, loss of myelinated fibers and signs of regeneration and necrotic fibers. All symptoms ameliorated with the initiation of a gluten-free diet. A repeat muscle biopsy only showed residual, mild myopathic features.

A muscle biopsy in a case of CD and myositis described by Alawneh et al. revealed muscle necrosis with neutrophilic infiltration with secondary leukocytoclastic vasculitis consistent with neutrophilic myositis [31].

A large case series of thirteen patients with gluten-related myopathy was published in 2007 [32]. The main pathological findings on muscle biopsy included internalization of nuclei, basophilic rimmed

vacuoles and fiber splitting, endomysial chronic inflammatory cell infiltrate (CD3+ cells) (Figure 4) and signs of fibrosis in seven cases.
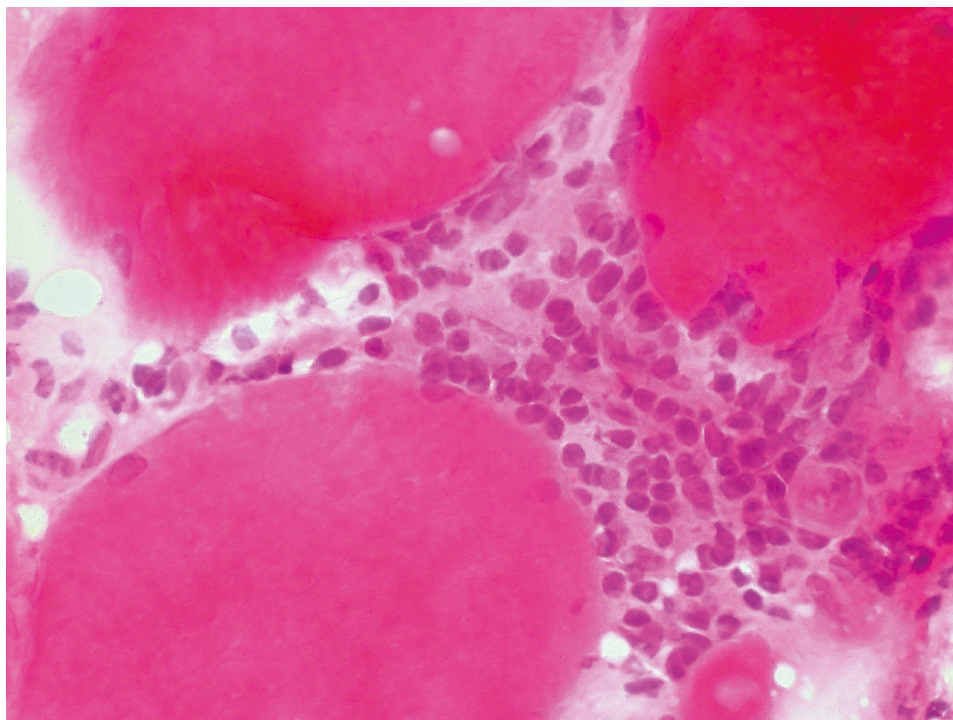
Figure 4. Skeletal muscle biopsy obtained from a patient with gluten myopathy showing myocytes surrounded by lymphocytic infiltration suggestive of an inflammatory process (H&E stain, magnification 400×).

3.6. Encephalopathy and Gluten Sensitivity

Encephalopathy is a clinical term implying global brain dysfunction. Patients with encephalopathy can have a spectrum of symptoms ranging from headaches, confusion, disorientation, cognitive deficits, slow mentation and, in extreme cases, altered level of consciousness.

Brücke described a case of a 45-year-old male with progressive loss of energy, arrhythmic myoclonic movements of the arms and tongue, dysarthria, facial palsy and concurrent diagnosis of CD [33]. The patient died a year later from a severe pulmonary embolism. Post-mortem examination revealed brain edema and periventricular lesions with inflammatory necrosis and myelin loss. The inferior olives were hypertrophic and there was gliosis in the vermis with lymphocyte infiltration in the pons and mesencephalon.

Keller and Dimberg presented 2 cases of refractory CD and encephalopathy [34,35]. Refractory CD is defined by persisting malabsorption and villous atrophy despite strict adherence to a gluten-free diet [36]. The neurological symptoms were accompanied by gastrointestinal symptoms characterized by severe diarrhea and weight loss, both patients died within four months after the onset of neurological complaints. Neuropathological findings included loss of Purkinje cells, neuronal loss of the dentate nucleus and perivascular cuffing of lymphocytes. The pons, midbrain, thalamus and basal ganglia showed arterial changes and mild degeneration of the pyramidal tract and posterior columns with loss of myelinated nerve fibers. In the case described by Dimberg neuropathological examination

also revealed widespread perivascular lymphocytosis in the cortex hippocampus and temporal gyrus [34,35].

Hu and colleagues reported on thirteen patients with amnesia, personality change, confusion, disorientation, ataxia and seizures who also had CD [37]. Two patients underwent frontal lobe biopsy and three patients underwent post-mortem examinations. These revealed non-specific gliosis and astrocytosis. In a 57-year-old male ubiquitin-positive inclusions were found.

La Mantia et al. reported a case of a female patient with intermittent headaches that progressed into chronic headache at the age of 29 years [38]. She was hospitalized five years later with papilledema, peri-papillary hemorrhages and brain swelling. A lobectomy was performed because of a severe intracranial hypertension. Examination of the resected tissue revealed calcifications, increased vascularity, mild neuronal loss, reactive gliosis and demyelination and edematous changes. Coeliac disease was diagnosed at the same time. After the initiation of a gluten-free diet she improved dramatically and was able to resume her job as a teacher. Figure 5 illustrates the infiltration of lymphocytes in the cerebral tissue of a patient that died of gluten encephalopathy.
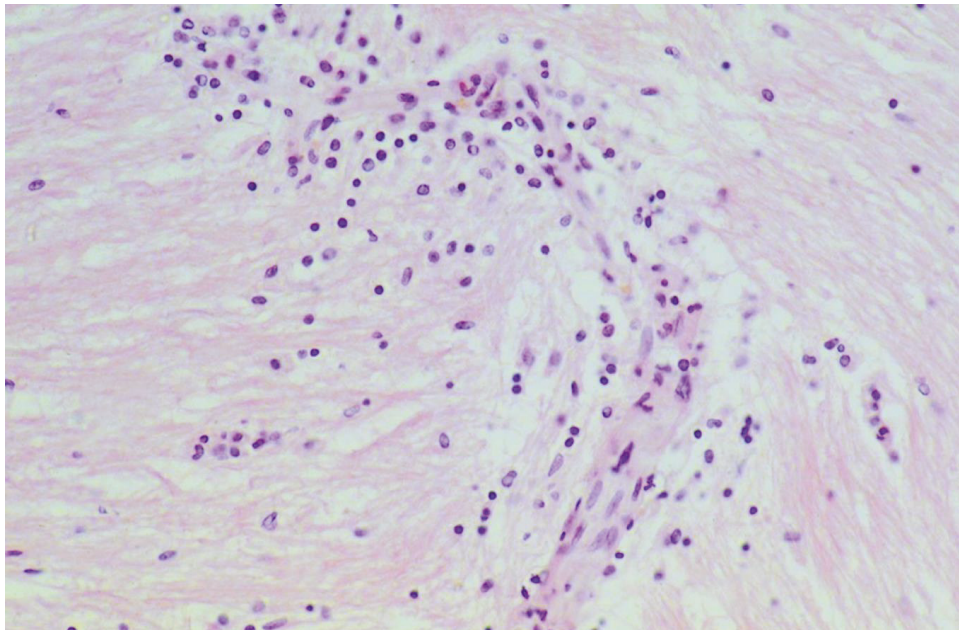
Figure 5. Postmortem cerebral white matter tissue from a patient with gluten encephalopathy showing infiltration with inflammatory lymphocytes and perivascular cuffing (H&E staining, magnification 100×).

3.7. Epilepsy and Gluten Sensitivity

A wide range of diseases can manifest with seizures [39]. Coeliac disease has been linked to epilepsy. CD is two times more prevalent in epilepsy patients compared to the general population and epilepsy is 1.8 times more prevalent in CD [40]. In some pediatric cases, occipital calcifications can be identified on brain imaging. This combination is termed coeliac disease, epilepsy and cerebral calcifications (CEC) [41].

Two papers have provided neuropathological findings in this group of patients. Bye et al. describe a female patient with CEC and folate deficiency [42]. Her first seizures started at the age of four and worsened in her puberty. Diagnostic evaluation revealed persistent iron and folate deficiency. Subsequently CD was diagnosed, and she started a strict gluten-free diet. An extensive resection of

the right lateral occipital cortex was performed. Macroscopically pial angiomatosis was observed, consisting of groups of small veins entrapped by collagen. Severe sclerosis of veins and lymphocytic perivascular cuffing was observed in the cortical neuropil. There was atrophy of the white matter and influx of macrophages, with reactive gliosis. A similar case was reported on a girl with macrocephaly, epilepsy and autistic traits [43]. The girl started having epileptic seizures with episodes of apnea at the age of five months. The seizures gradually developed into generalized complex seizures. At the age of two years CD was diagnosed. She died three years later, probably following an epileptic attack. Autopsy revealed no specific abnormalities apart from megalencephaly.

4. Discussion

This systematic review examined the neuropathological findings in gluten-related neurological disorders. Neuropathological findings in the context of gluten ataxia showed loss of Purkinje cells, cerebellar atrophy and gliosis, especially in the granular layer with also atrophy of the dentate nucleus. However, findings were not limited to the cerebellum, but involved other parts of the central nervous system that are closely linked to the cerebellum such as the pons, inferior olives and thalamus.

While vitamin B1, B3, B6, B12, E deficiencies are well known causes of neuropathy and other neurological disorders and can be a consequence of malabsorption due to untreated CD, they are unlikely to be responsible for the pathology described in this review. Furthermore, in almost all cases discussed in this review other possible causes for the neurological deficit, like genetic causes had been ruled out. This review suggests that the pathophysiology of neurological damage in the context of gluten sensitivity has an immune mediated basis.

Whilst there is a female predominance in CD (2.4 F:1.M) and other auto-immune diseases [44], the majority of gluten-related neurological disorders affected men (57%). In the ataxia group this percentage was even higher (76%). The median age at onset of neurological complaints was 50.3 years (excluding the two epilepsy cases with onset at age 6 months and 5 years). The median age at time of CD diagnosis was 44.9 years. There is an increased risk of autoimmunity in individuals diagnosed with CD later on in life [45]. Whether this increased risk can be attributed merely to age and years of gluten exposure is still debated [46,47].

More importantly, in most studies, adherence to a strict gluten-free diet was not monitored. It is therefore unknown whether patients were still exposed to gluten at the time of the development of their neurological dysfunction. [48–53].

A consistent finding in three of the ataxia studies was the presence of diffuse infiltrates and perivascular cuffing with lymphocytes in the cerebellar tissue [9,14,15,18]. Lymphocytic infiltration was also demonstrated in several nerve biopsies of patients with neuropathy [17,22,25], muscle biopsies of myopathy patients [26,27,30,32] and in brain tissue of patients with encephalopathy [33,35] and epilepsy [42]. Characterization of these cell infiltrates was only performed by Mittelbronn et al. [15] who described a cytotoxic T-cell population (CD8+ and granzyme B+), and by Souayah (CD68+/CD45ro+ cell populations) [22]. The origin and target epitope of these lymphocytes remain unclear. However, these findings strongly support the notion that the pathology is immune mediated and not related to vitamin or trace elements deficiencies.

It is as yet unknown if and how such cells travel from the intestine to the brain. Crossing of the blood-brain barrier may occur after a compromise due to local inflammation [54]. Nanri et al. [16] hypothesized a humoral response in which anti-gliadin antibodies also recognize epitopes on Purkinje cells. Indeed Hadjivassiliou et al. have demonstrated that anti-gliadin antibodies cross-react with Purkinje cells in vitro. Previously published work has also shown that sera of gluten ataxia patients strongly stain Purkinje cells in cerebellar rat tissue, even after adsorption with crude gliadin [55]. In another study the sera of twenty CD patients and twenty healthy controls were applied on rat brain sections. Sixteen CD patient sera showed immune-reactivity for IgA or IgG on Purkinje cells, deep cerebellar nuclei and brainstem neurons whereas only four sera from healthy controls showed immune-reactivity on the rat brain sections. An additional adsorption experiment with recombinant

Transglutaminase (TG2) indicated that anti-TG2 antibodies substantially contribute to neuronal epitope recognition. Of interest is that injection of these antibodies into the lateral ventricle of mice resulted in motor dysfunction. The IgA component from the CD patient sera also cross-reacted with Transglutaminase 3 and 6 [56].

Transglutaminase 6 (TG6), a member of the Transglutaminase family of protein-crosslinking enzymes and is closely linked to TG2 (the autoantigen in CD) and Transglutaminase 3 (TG3, the autoantigen in dermatitis herpetiformis). Transglutaminase 6 has been proposed as the autoantigen in gluten-related neurological disorders [57,58]. IgA deposits against TG6 have been observed in vessels from cerebellar tissue of a patient with gluten ataxia [49]. Moreover, antibodies against TG6 have been detected in the sera of patients with gluten ataxia (73%) and gluten neuropathy (50%), regardless of enteropathy [59,60]. These studies indicate that TG6 antibodies might be helpful in the diagnostic workup of GRND.

The current gold standard for CD diagnosis is based on Transglutaminase 2 (TG2) antibodies (with or without additional testing for endomysium antibodies) followed by histological examination of duodenal biopsies (presence of villous atrophy, crypt hyperplasia and increased intraepithelial lymphocytes). However, even if clinicians consider GRND in a patient with idiopathic neurological complaints, the diagnostic yield using these antibodies is low. This is because the majority of patients with GRND are seronegative for TG2 because they do not have enteropathy [48,49]. Therefore a TG2 antibody test is not sufficient to diagnose GRND [50]. Recent data suggest that a coeliac "lymphogram", defined as an increase in CD3+ T-cell receptor gamma delta+ (TCRγδ+) intraepithelial lymphocytes (IEL) plus a concomitant decrease in CD3− cells in a mucosal duodenal biopsy, was associated with a sensitivity of 87% (CI, 73.7–95%) and specificity of 96.7% (82.7–99.9%) for CD [52,53]. Therefore it might be worthwhile to assess the intraepithelial lymphogram of duodenal biopsies in (suspected) GRND patients that test negative for TG2 but are positive for TG6 and gliadin antibodies.

Addolorato and colleagues observed a state of hypo-perfusion in multiple cerebral brain regions in untreated CD patients compared to gender and age-matched CD patients on a gluten-free diet and healthy control subjects [61]. In another study, significantly more perfusion abnormalities in the frontal cortex were found in CD patients, regardless of dietary regimen, compared to gender- and age-matched controls [62]. It is unclear if these findings have a bearing on the cognitive deficits observed in patients with gluten encephalopathy.

Three patients, described by Keller, Dimberg (gluten encephalopathy) and Souayah (small-fiber neuropathy), suffering from RCD [22,34,35] had evidence of aberrant intraepithelial lymphocytes that disseminated into mesenteric lymph nodes, blood, bone marrow and other epithelial tissue like skin or lung [63]. It is possible that these aberrant T-cells can also enter the central nervous system. This may explain the observation that ataxia with cortical myoclonus can be a phenotype of RCD that is often extremely difficult to treat both from the gut and the brain perspective. Whatever seems to be driving the gut inflammation seems to also be driving the brain inflammation generating the disabling cortical myoclonus and the ataxia.

Future studies should focus on the characterization of lymphocytes found in brain tissue or CSF and clonality studies may be able to clarify if they originated from the gut. In addition investigating the role of TG6 as a target autoantigen in neurological manifestations may shed light to the pathophysiology of GRND.

5. Conclusions

The neuropathological findings in gluten-related neurological disorders are widespread and not limited the cerebellum.

Information on the nature of the lymphocytic infiltration is lacking.

The current evidence is suggestive of both humoral and cell-mediated immunological responses.

More research is needed to further investigate the underlying neuropathological mechanism by characterization of the inflammatory cell infiltrate and identification of target epitopes.

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Article

Cognitive Impairment in Coeliac Disease with Respect to Disease Duration and Gluten-Free Diet Adherence: A Pilot Study

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Abstract: Cognitive deficit has been reported in coeliac disease (CD), but previous reports often study heterogenous samples of patients at multiple stages of the disease, or lack control data. Healthy controls ($N = 21$), newly diagnosed CD patients (NCD; $N = 19$) and established CD patients (ECD; $N = 35$) were recruited from a specialist UK centre. Participants underwent a cognitive test battery that established seven overall domain scores. The SF-36 was administered as a quality of life (QoL) measure. Controlling for age, data were compared in between-group ANCOVAs with Tukey's post-hoc test. Any significant outcome was compared in the ECD group only, between patients who were gluten-free diet adherent vs. non-adherent (defined via Biagi score and serology results). NCD and ECD groups underperformed relative to controls, by comparable degrees, in visual (overall model: $p < 0.001$) and verbal ($p = 0.046$) memory. The ECD group only underperformed in visuoconstructive abilities ($p = 0.050$). Regarding QoL, the NCD group reported lower vitality ($p = 0.030$), while the ECD group reported more bodily pain ($p = 0.009$). Comparisons based on dietary adherence were non-significant. These findings confirm cognitive deficit in CD. Dysfunction appears established at the point of diagnosis, after which it (predominantly) stabilises. While a beneficial effect of dietary treatment is therefore implied, future research is needed to establish to what extent any further decline is due to gluten exposure.

Keywords: coeliac disease; cognition; neurology; gluten-free diet; disease duration

1. Introduction

Coeliac disease (CD) is an autoimmune disorder triggered by ingestion of gluten [1]. CD is a global problem, with a current estimated prevalence of 1% in most European countries [2]. Currently, the only treatment is strict adherence to a gluten-free diet.

Patients suffering with CD can have an abnormal reaction to gluten that classically causes gastrointestinal symptoms such as abdominal pain, diarrhoea, constipation, bloating and weight loss (sometimes referred to as classic CD). Yet some patients may not present with any gastrointestinal symptoms but may have extraintestinal manifestations. It is now accepted that CD is a multi-system disorder and can be associated with anaemia, osteoporosis, liver disease and skin manifestations [3,4]. Furthermore, there is increasing evidence that CD can present with neurological problems such as cerebellar ataxia, peripheral neuropathy and encephalopathy [5].

Cognitive deficits are often reported by patients with CD. CD has been associated with 'brain fog,' which refers to a range of symptoms including cognitive slowing, difficulty concentrating and problems with short- and long-term memory. One study found that patients with gluten ataxia showed significant verbal memory deficits [6]. Recent recognition of the role of the cerebellum in cognitive functions (cerebellar cognitive affective syndrome) could potentially explain some of these deficits, given that the cerebellum is very frequently the target organ in the context of CD [7]. Furthermore, gluten encephalopathy encompasses patients with such cognitive deficits, sometimes associated with frequent headaches and an abnormal MRI scan showing excessive white matter changes over and above those associated with aging [8]. It is currently unclear what the clinical implications of these brain abnormalities are for CD patients. A large epidemiological study found that patients with CD were at an increased risk of vascular dementia [9]. A possible relationship between early onset dementia and CD has also been identified, with patients showing moderate to severe intellectual deterioration. Diffuse cerebral or cerebellar atrophy was also detected on CT scans [10].

An early study by Hallert and Astrom [11] investigated a series of newly diagnosed, consecutive CD patients and found borderline cognitive impairment in 21% of cases. Contemporary research investigating this is relatively scarce but has continued to produce positive findings. One study found that CD patients who had been treated for an average of 5.5 years performed worse than controls on tests examining executive functioning and processing speed [12]. Another has indicated that some cognitive functions, also including visual memory, improve in patients after consuming a gluten-free diet (GFD) for a year [13]. Finally, a recent study confirmed the presence of a reaction time deficit compared to controls in a cohort of CD patients taken from a national UK database [14]. However, findings such as these have also been refuted by a study that found no evidence of cognitive impairment in a group of 28 newly diagnosed CD patients [15]. It should be noted that this study potentially lacked experimental sensitivity, as it only used a very brief screening measure of cognitive functioning. Furthermore, they compared the performance of CD patients with a control group of patients who presented to their gastroenterology clinic with Coeliac-like symptoms but in whom CD was ruled out. The authors did not comment on the aetiology of the symptomatic control group and they did not compare performance with a healthy control sample. As such, the conclusion of no cognitive impairment is questionable.

Another limitation of such studies that look at cognitive dysfunction in patients with established CD is that very few control for the effect of disease stage/treatment. The one aforementioned study which examined cognitive change over time from the point of diagnosis indicated a degree of recovery [13], suggesting this to be an important variable to consider. A GFD has otherwise been established as beneficial for patients with gluten ataxia, gluten encephalopathy and gluten neuropathy [16]. Therefore, it is imperative that future studies on patients with CD stratify based on whether the condition is newly diagnosed or established. Further, no study has both examined cognitive change over time and simultaneously included control data to ascertain how any scores deviate from a baseline level of expected performance.

To further understand the prevalence of cognitive problems in CD and the impact of a gluten-free diet, the current study collected pilot data on patients with CD at different disease stages to address the following three main questions:

(1) Do newly diagnosed patients with CD have cognitive difficulties when compared to healthy controls?

(2) Do patients with a longstanding diagnosis of CD differ from newly diagnosed patients with regard to their cognitive profile?

(3) Do patients who comply with a gluten-free diet have better cognition than those who do not?

2. Materials and Methods

2.1. Participants

CD participants were recruited from a specialist coeliac disease clinic held at Sheffield Teaching Hospitals NHS Trust from September 2015 to July 2016. To be eligible for this study, all participants had to be aged between 18 and 70 years and be proficient in English language. It was important to include a wide age range, as CD can be diagnosed at any age [17]. To be included in the CD groups, their diagnosis had to be based on a small bowel biopsy histology in keeping with CD (triad of villus atrophy, crypt hyperplasia and increased intraepithelial lymphocytes) taken at baseline.

Patients with newly diagnosed CD were approached consecutively during this period when they attended their clinic appointment post-biopsy result. A second group of patients with established CD (had held their diagnosis for at least 5 years) were identified from a database at the same clinic and invited to take part by the study team. This established group was further separated into those who were gluten-free diet adherent and those who were not. This was determined by the participant's responses on the Biagi scale [18] and their last clinical serological analysis for gliadin antibodies (which they receive as part of routine clinical care; ELISA assay, Phadia 2500) in combination with the local clinical cut-offs. Being adherent to the diet was defined as being both adherent according to Biagi score and serological negativity.

The control participants were a convenience sample including friends and family of patients attending the clinic. These subjects did not have a diagnosis of CD or gluten sensitivity and were not following a GFD. The project investigators intended to match the control group with the CD patients as closely as possible in terms of age, gender, years of education and estimated pre-morbid functioning. Participants in all groups were excluded if they had any known neurological conditions, psychiatric conditions and a history of substance abuse. Participation in this study was voluntary. However, travel expenses were available. This study was approved by the South West Cornwall and Plymouth Regional Ethics Committee (REC reference; 15/SW/0096, 10/04/2015). All participants provided written informed consent.

The overall patient recruitment process is visualised in Figure 1. The healthy controls ($N = 21$) had a mean(SD) age of 43.5 (16.2), and were 76.2% female. The newly diagnosed CD cases ($N = 19$) were aged 45.1 (17.3) and were 84.2% female. The overall established CD group ($N = 35$) had a mean(SD) age of 55.5 (12.7), were 88.6% female and were a mean of 11.8 years post-diagnosis (range: 5.2–45.1, SD = 7.8). Of these, 16 were determined to be dietary adherent while 19 were not.
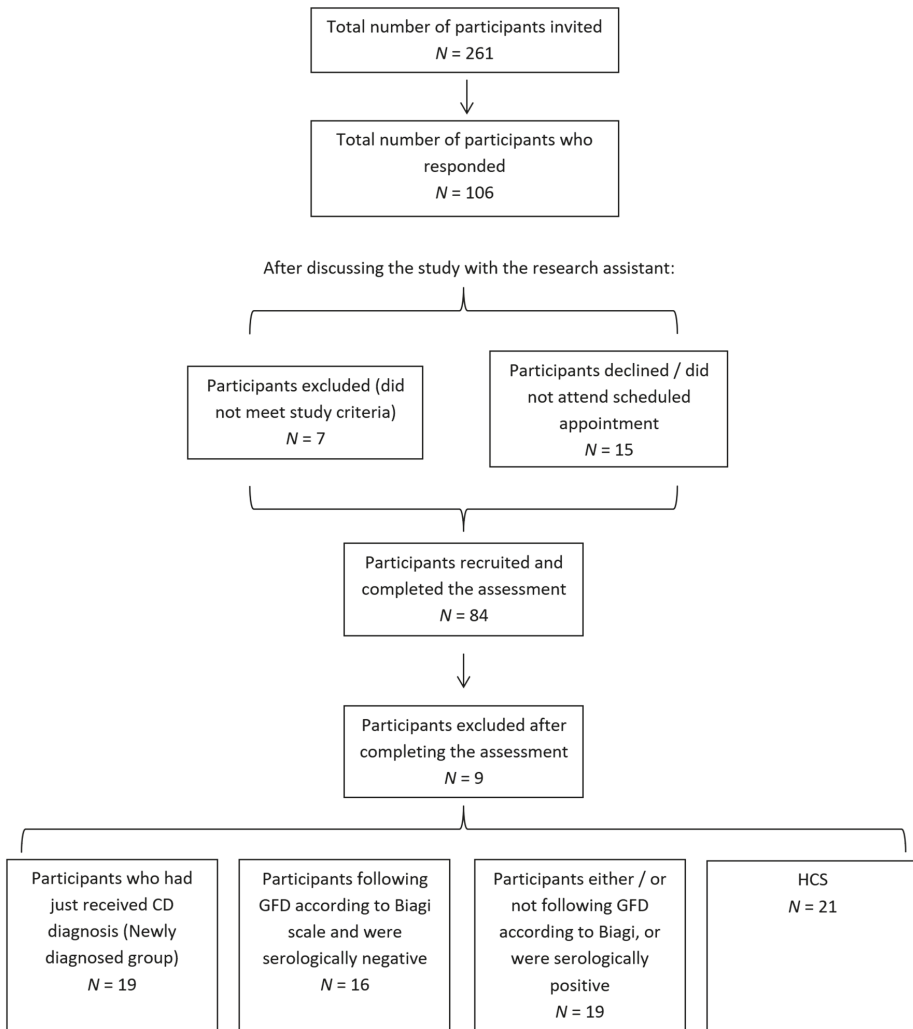
Figure 1. Participant recruitment process, including sample sizes. HCS, healthy control subjects.

2.2. Study Power

This analysis is presented as a pilot study. Nonetheless, previous comparable investigations of cognitive outcomes in CD have used sample sizes which are smaller than those in the current analysis. Casella et al. [12] used two groups of 18, while Lichtwark et al. [13] used a repeated-measures design on a single group of 11. Each of these papers reported significant findings in outcomes from cognitive testing, indicating that they were sufficiently powered to detect experimental effects.

2.3. Design

This study followed a cross-sectional design to confirm or reject the presence of cognitive deficits at different stages of CD.

2.4. Assessment Procedure

Participants attended one appointment for 2 h with the research assistant where written consent was provided. The newly CD diagnosed participants had to be tested within 4 weeks of receiving their diagnosis. All participants completed the same neuropsychological assessments in a consistent order to ensure that the delay conditions were adhered to. All of the assessments consisted of standardised clinical instruments, administered according to the standardised instructions provided by the assessment manuals. Quality of life measures were also included to investigate the relationship between gluten-free diet adherence, symptomatology and cognitive difficulties. Any participant who did not complete all outcomes was excluded from analysis.

2.5. Testing Battery and Initial Data Handling

The cognitive test battery included (1) the Test of Premorbid Functioning (ToPF); (2) the Wechsler Adult Intelligence Scale-III (WAIS) tests of block design, vocabulary, matrix reasoning and similarities; (3) Trail Making Test (TMT); (4) Controlled Oral Word Association Test (letter fluency only, COWAT); (5) Digit Span; (6) story recall; (7) California Verbal Learning Test (CVLT); (8) Rey–Osterrieth Complex Figure Test (CFT); (9) Digit-Symbol Coding; (10) Speed of Information Processing (SoIP); (11) Boston Diagnostic Aphasia Examination for Verbal Agility (BDAE Verbal Agility) [19].

Key scores for each test were identified according to common convention. On examination, it was found that the BDAE Verbal Agility total score exhibited a strong ceiling effect, wherein the vast majority of participants scored maximum points. It was therefore decided to ignore this outcome in the main analyses to maintain experimental sensitivity. The ToPF IQ was calculated so that experimental groups could be compared on this. Otherwise, all cognitive variables were converted to Z scores relative to the performance mean and standard deviation of the control group. It was further ensured that all outcomes were transformed (if needed) such that a higher score always represented a better performance.

Specific outcomes were then averaged, with equal weighting, into seven overall cognitive "domain" scores. Their names and composition were as follows: mental flexibility—raw scores from WAIS block design and matrix reasoning, TMT ("B" condition as a percentage of "A" condition) and COWAT letter fluency; visuoconstructive abilities—WAIS block design raw score and CFT copy condition; verbal ability—raw scores from WAIS vocabulary and similarities; overall verbal memory—immediate and delayed scores from story recall, and from the CVLT immediate free recall, delayed free recall, recognition "hits" and recognition "false positives"; overall visual memory—immediate and delayed scores from the CFT; working memory—Digit Span backwards; processing speed—TMT ("A" condition), Digit-Symbol Coding raw score and SoIP motor-adjusted score.

Subdomains were also constructed for overall verbal memory and overall visual memory, such that short-term (ST) and long-term (LT) components of these may be separately examined (e.g., LT verbal memory would specifically include "delayed" verbal memory tasks and the "recognition" portion of the CVLT).

For quality of life (QoL), the SF-36 was used [20]. Raw scores from this were converted to the recommended eight outcomes for physical functioning, physical roles, bodily pain, vitality, general health, social functioning, emotional roles and mental health.

2.6. Statistical Analysis

Analyses were performed using SPSS Version 25.

All variables were visually inspected for normality to determine what form of testing should be used. A non-normal distribution would mean that the appropriate non-parametric test would be used in cases examining only a single outcome. However, for analyses with an additional controlling covariate (e.g., age), an ANCOVA was performed and the distribution of residuals/error were inspected for normality to confirm that the model assumptions had not been broken.

Major groups (controls/newly diagnosed cases/all established cases) were statistically compared on age, sex, years of education and ToPF IQ (univariate ANOVAs/chi-squared analyses). Any variable where significant differences were found would be used as a covariate in relevant analyses. Established CD subgroups (i.e., based on dietary adherence) were also compared on these variables, which would then be used as a covariates in the same manner.

For all cognitive domain and QoL outcomes, the three major groups were compared by univariate ANOVA. Significant findings from these initial analyses would justify further post-hoc testing. Tukey's post-hoc test was used to confirm the specific groups where the differences lay. Further, if differences were found in either overall visual/verbal memory cognitive domains, then the ST and LT subdomains of these were also compared in the same manner, with additional testing being conducted as appropriate to determine whether a LT deficit was unique, or a consequence of an accompanying ST deficit.

Established CD dietary adherence subgroups were compared to one another on any variable where a significant difference was reported in these primary comparisons.

Finally, if justified by other findings, correlations between significant cognitive and QoL outcomes would be considered.

3. Results

3.1. Analyses of Samples' Demographics

Groups were significantly different for age (ANOVA; $p = 0.007$), wherein the established CD cases were older than both newly diagnosed cases ($p = 0.044$) and controls ($p = 0.013$), who were not themselves different from one another. Otherwise there were no significant differences for any other "demographic" variable, including ToPF IQ. Established CD subgroups based on dietary adherence were also not significantly different from one another on any of these variables, or the number of years since their diagnosis. Full details are available in Table 1.

Table 1. Demographics of the study groups.

Variable	HCS	Newly Diagnosed	All Established Cases	Results of Statistical Comparison between Major Groups	Subgroup: Established CD and Diet Adherent	Subgroup: Established Case and Not Diet Adherent	Results of Test Comparing Established CD Subgroups
Sample Size	21	19	35	-	16	19	-
Age	43.5 ± 16.2	45.1 ± 17.3	55.5 ± 12.7	$p = 0.007$ [1] F = 5.329	57.8 ± 10.6	53.6 ± 14.3	$p = 0.481$
% Female	76.2% (N = 16)	84.2% (N = 16)	88.6 (N = 31)	$p = 0.473$	93.8% (N = 15)	84.2% (N = 16)	$p = 0.377$
Years of Education	15.2 ± 2.0	14.1 ± 3.0	13.8 ± 3.0	$p = 0.172$ F = 1.802	14.2 ± 3.2	13.4 ± 2.8	$p = 0.452$
ToPF IQ	106.4 ± 5.6	104.4 ± 8.8	104.8 ± 8.2	$p = 0.662$ F = 0.415	106.6 ± 6.8	103.3 ± 9.1	$p = 0.239$
Years since coeliac diagnosis	-	0.0	11.8 ± 7.8	-	11.4 ± 4.4	12.1 ± 9.8	$p = 0.803$

[1] Established CD cases were significantly older than both the newly diagnosed group ($p = 0.044$) and controls ($p = 0.013$). Data are the means and standard deviations. HCS, healthy control subjects.

3.2. Analyses of Cognitive Performance

ANCOVAs controlling for age revealed that groups were significantly different from one another in visuoconstructive abilities ($p = 0.050$), overall verbal memory ($p = 0.046$) and overall visual memory ($p < 0.001$) domains. Tukey's post-hoc test determined that in the case of visuoconstructive ability, the group difference was driven by established CD cases performing significantly worse than both newly diagnosed cases and controls (who were not themselves different from one another). However, in the case of both overall visual and verbal memory domains, the significant effect was driven by

established and newly diagnosed groups performing significantly worse than controls (while not themselves being different from one another). Full details are presented in Table 2 and Figure 2.

Table 2. Results of groups comparisons (ANCOVAs, controlling for age) on the cognitive domain scores.

Cognitive Outcome	HCS Mean ± SD	Newly Diagnosed Mean ± SD	Established CD Mean ± SD	p Value F Value
Mental Flexibility	0.00 ± 0.60	−0.14 ± 1.05	−0.80 ± 0.95	p = 0.064 F = 2.850
Visuoconstructive Ability	0.00 ± 0.79	−0.11 ± 1.38	−0.91 ± 1.09	**p = 0.050** [1] F = 3.130
Verbal Ability	0.00 ± 0.87	−0.07 ± 1.67	−0.59 ± 1.03	p = 0.129 F = 2.105
Overall Verbal Memory	0.00 ±0.83	−0.97 ± 1.65	−0.93 ± 1.19	**p = 0.046** [2] F = 3.216
Overall Visual Memory	0.00 ± 0.96	−2.04 ± 2.40	−2.49 ± 2.02	**p < 0.001** [3] F = 8.517
Working Memory	0.00 ± 1.02	−0.26 ± 1.51	−0.18 ± 1.66	p = 0.762 F = 0.273
Processing Speed	0.00 ± 0.74	0.31 ± 1.02	−0.13 ± 1.03	p = 0.332 F = 1.121

[1] For visuoconstructive ability, established CD cases performed worse than newly diagnosed (*p* = 0.034) and control groups (*p* = 0.010). [2] For overall verbal memory, established (*p* = 0.021) and newly diagnosed (*p* = 0.042) groups performed worse than controls. [3] For overall visual memory, established (*p* < 0.001) and newly diagnosed (*p* = 0.003) groups performed worse than controls. HCS, healthy control subjects.
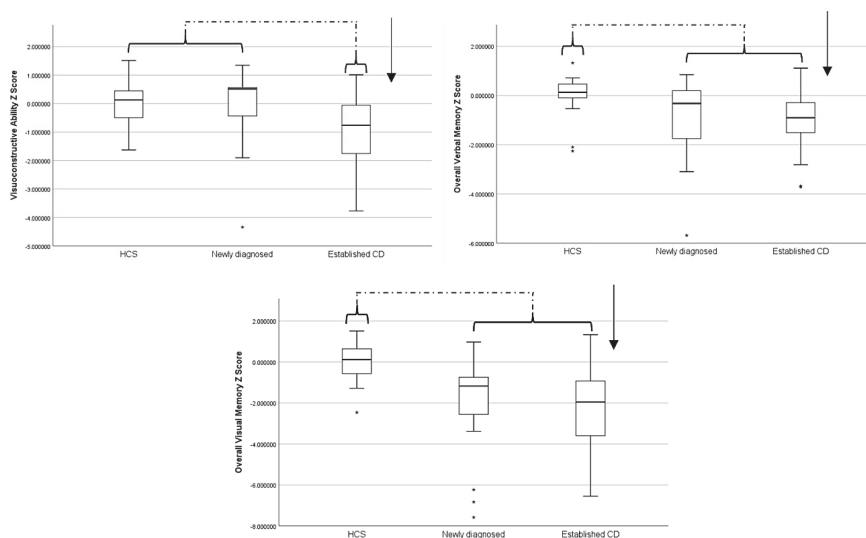
Figure 2. Boxplots visualising group performance on cognitive domains found to be significantly different in statistical analyses. Asterisks represent data points identified as outliers by the statistical software. Brackets and arrows are superimposed to demonstrate the significant difference between groups, with reference to control group performance, within each model. HCS, healthy control subjects.

Further analysis on the long-term (LT) and short-term (ST) components of the verbal and visual memory domains revealed that the verbal memory finding had been driven by a worsening of the LT memory subcomponent specifically (*p* = 0.019), and that the ST memory subcomponent did not exhibit any significant effects (Table 3). However, both ST (*p* < 0.001) and LT (*p* = 0.002) visual memory subcomponents had worsened in CD cases. Tukey's post-hoc test confirmed that the between-group pattern of findings was not different in these subdomains compared to the parent analyses. A further ANCOVA was conducted to control for ST visual performance in explaining the LT visual outcome

(i.e., ST domain scores were included as a covariate in addition to age and group). Here, the study group did not retain its significance ($p = 0.631$), while ST performance was highly significant ($p < 0.001$).

Table 3. Results of additional analyses comparing short-term (ST) and long-term (LT) components of visual and verbal memory between groups.

Cognitive Outcome	HCS Mean ± SD	Newly Diagnosed Mean ± SD	Established CD Mean ± SD	p Value F Value
ST Verbal Memory	0.00 ± 0.59	−0.29 ± 0.80	−0.29 ± 0.74	**p = 0.478** F = 0.745
LT Verbal Memory	0.00 ± 0.85	−1.23 ± 1.91	−1.18 ± 1.34	**p = 0.019** F = 4.206
ST Visual Memory	0.00 ± 1.02	−2.13 ± 2.31	−2.65 ± 2.09	**p < 0.001** F = 9.472
LT Visual Memory	0.00 ± 1.02	−1.95 ± 2.51	−2.33 ± 2.01	**p = 0.002** F = 7.089

Tukey's post-hoc test confirmed that the pattern of between-group differences replicated the corresponding analyses concerning overall visual and verbal memory domains. HCS, healthy control subjects.

In the analysis of the SF-36 scores, significant effects were observed for items assessing bodily pain ($p = 0.009$) and vitality ($p = 0.030$, Table 4). Tukey's post-hoc test found that the bodily pain result was driven by established CD cases ($44.14 ± 11.56$) having a worse outcome than controls ($54.39 ± 10.51$). However, the vitality result was driven by newly diagnosed CD cases ($47.83 ± 10.24$) having a worse outcome than controls ($55.16 ± 5.81$).

Table 4. Results of "major group" ANCOVA analyses (controlling for age) comparing outcomes from the SF-36 between controls, newly diagnosed CD cases and established CD cases.

SF-36 Outcome	HCS Mean ± SD	Newly Diagnosed Mean ± SD	Established CD Mean ± SD	Model Results
Physical Functioning	51.33 ± 10.21	51.50 ± 8.24	47.49 ± 10.18	p = 0.732 F = 0.313
Physical Role	52.85 ± 7.26	50.64 ± 10.40	46.93 ± 10.10	p = 0.204 F = 1.627
Bodily Pain	54.39 ± 10.51	50.82 ± 9.06	44.14 ± 11.56	**p = 0.009** F = 4.977
General Health	50.51 ± 6.21	46.91 ± 9.01	44.80 ± 10.33	p = 0.127 F = 2.126
Vitality	55.16 ± 5.81	47.83 ± 10.24	51.10 ± 8.67	**p = 0.030** F = 3.700
Social Functioning	54.27 ± 4.71	48.85 ± 7.83	49.06 ± 9.60	p = 0.094 F = 2.449
Emotional Role	51.80 ± 9.01	48.66 ± 11.21	51.10 ± 8.14	p = 0.534 F = 0.632
Mental Health	50.44 ± 6.89	49.14 ± 10.37	50.90 ± 9.69	p = 0.841 F = 0.173

Significant differences were found in bodily pain, where established CD cases have worse scores than HCS ($p = 0.002$), and vitality, where newly diagnosed cases have worse scores than HCS ($p = 0.021$). HCS, healthy control subjects.

Testing was performed to compare dietary adherence subgroups within the established CD cases on all variables where a significant effect was found in the major group analyses as described above. However, no significant subgroup differences emerged.

Correlation analyses were conducted separately within each experimental group for a provisional investigation of any relationships between cognitive and QoL variables which had been found to be significant in the above analyses. However, no significant effects were observed. Finally, an alternative means of controlling for age differences between major groups was implemented. Significant between-group findings (i.e., verbal/visual memory, and visuoconstructive ability) were

re-analysed via independent samples *t*-tests using subsamples of the control (now $N = 10$, age = 55.4) and newly diagnosed (now $N = 12$, age = 55.7) groups, which were age-matched against the original, established CD group. These analyses all retained significance and the original pattern of difference.

4. Discussion

In this study, the impact of CD on cognitive performance and QoL was assessed with respect to duration of illness and dietary success. Both newly diagnosed CD and established CD patients were found to underperform compared to controls in tests investigating verbal and visual memory. Established CD cases were also found to underperform compared to both newly diagnosed cases and controls in measures of visuoconstructive ability. Further, worse QoL outcomes were variably observed between patient groups, with newly diagnosed patients specifically reporting lower vitality and established patients specifically reporting worse bodily pain. Comparisons of these preliminarily significant variables in subgroups of established patients based on their dietary adherence did not reveal any significant results. In addition, no significant correlation was found between cognitive scores and QoL measures.

Cognitive deficit in CD has been established in limited previous studies [11–14]. In addition to evidence of amnesia, acalculia, confusion and personality change, the cognitive domains reported to be affected from these include components of executive functioning, processing speed and memory. Other case and small group studies on patients with CD presenting with cognitive dysfunction have shown variable responses to a GFD [10,21,22], although these relied on clinical cognitive assessment reports, which may be relatively insensitive at detection of more "subtle" forms of deficit [23]. Overall, this past research has lacked investigations which simultaneously consider the potential beneficial effect of a GFD over time, and which include control data to establish a meaningful performance baseline against.

The primary analyses of the current study considered cognitive performance between a control group and two groups of CD patients based on the stage of their disease—newly diagnosed or established. In these comparisons, CD groups were observed to underperform in three of the seven domains investigated. For visual and verbal memory domains, post-hoc testing indicated that the deficit was present in the newly diagnosed patients and had not appeared to worsen further when compared to established patients. Further analysis of these data found that these findings were driven by a long-term memory deficit in both domains, but a short-term deficit in only visual memory. Additional investigation of the visual memory outcomes confirmed that the underperformance in the long-term tests was likely driven by the accompanying short-term deficit rather than being a separate and unique consequence. CD patients also underperformed in tests of visuoconstructive abilities—except scores on this domain were comparable between controls and the newly diagnosed group, with the established cases specifically demonstrating a deficit. Taken together, these results demonstrate that cognitive deficit may be present at the earliest point of diagnosis but appears predominantly stable following treatment. The exception to this is the finding concerning visuoconstructive ability, which indicates that a degree of further decline may still be expected.

The affected domains in the current study show some agreement with previous literature; for visual memory, the Complex Figure Test was used, which was the same task that Lichtwark et al. observed patients with CD to improve on, following a year of a GFD [13]. However, while problems concerning mental flexibility and processing speed have been reported [12–14], these appear preserved in the current analysis. This disparity may be due in part to some of the specific tasks used differing between studies or issues of study power. Identification of a set of cognitive markers which are dependably affected in CD is highly desirable, and more research is required for this.

Circulating antibodies against gluten products are thought to be the primary cause of underlying brain pathology in gluten-related conditions. Transglutaminase 6 (TG6), an antibody common in gluten ataxia and also present in CD patients at a rate of approximately 40% [24], is expressed in a number of brain regions and particularly the cerebellum [25]. This may explain the characteristic patchy loss of cerebellar Purkinje cells observed in gluten ataxia [7]. Antibodies against gliadin are

ubiquitously produced in CD and have also been seen to exhibit reactivity with blood vessels which supply the brain [26]. This in turn may hypothetically lead to disruption of the blood–brain barrier and further downstream consequences such as vascular-driven white matter injury, which CD patients are increasingly seen to be at risk of [9]. CD also involves many nutritional consequences, and treated patients are at a raised risk of micronutrient deficiencies [27]. Some research has linked low levels of, e.g., folic acid [28] and vitamin D [29] to cognitive deficit. However, as routine nutritional monitoring is recommended by NICE guidelines for CD, it is unlikely that this would contribute to the findings of the current study.

Imaging studies of CD have been integral in characterising the brain tissue injury found in CD. Lowered brain volume in the cerebellum (whole cerebellar grey matter) and thalamus has been found in patients with TG6 positivity [24], while white matter changes affecting the tract connecting those regions (spinothalamic) in addition to the corpus callosum have been reported as a more general finding [14]. These brain locations are known to hold associations with the cognitive functions found to be impaired in the current study. The cerebellum is increasingly becoming recognised as playing an integral role in a range of cognitive functions, including ones underlying verbal and visual memory [30]. The thalamus is also reported to hold a number of associations with types of memory [31].

The finding of cognitive deficit in newly diagnosed patients agrees with previous research showing other neurological symptoms to be prevalent at the same stage in CD [24]. The pattern of our current results showing cognitive scores are often stable between new and established CD cases is also in general agreement with previous literature that a GFD is beneficial in preventing further neurological decline [13,16]. However, additional testing of established patient subgroups based on markers of their dietary adherence did not reveal any significant findings. These subgroups were defined by both their Biagi score and serological test results. Reliable assessment of GFD adherence is difficult, and measures of it (including those used in the current study) have been shown as lacking sensitivity [32]. It is possible that this issue, in combination with the diminished sample size for these analyses, contributed to a lack of findings. Dietary success is the foundation of CD treatment, and further cognitive research utilising more accurate methods of tracking is needed.

Quality of life was also examined, assessed by the SF-36 questionnaire. Significant findings here differed based on disease duration. Vitality was seen to be impacted in the newly diagnosed group. This is not necessarily surprising, given that this essentially describes energy/fatigue, which may still be impacted due to patients only recently having adopted a GFD. However, bodily pain was seen to be worsened in established patients only. While some research has shown that health-related QoL in CD is primarily dependent on dietary adherence [33], others have demonstrated the relationship may be more complicated and dependent on perceived, rather than actual, success [34]. Studies have also reported that sleep disorders are common in newly diagnosed CD patients and do not improve 1 year after following a GFD, and that this has a connection to depression, anxiety and fatigue [35]. In the current study, further analysis of the bodily pain result based on dietary compliance groups showed no significant findings. Due to the aforementioned issues of sensitivity in these groups, it should not be ruled out that this may still be due in part to, e.g., abdominal pain caused by ongoing gluten exposures among some of the patients. However, this also potentially supports such previous research showing that diminished QoL in CD is an ongoing problem that is relatively detached from engagement with a GFD.

This study had some limitations. The sample size, while comparable to other research investigating cognitive outcomes in CD [12,13], is relatively small. It is for this reason that we present the findings as pilot analyses and highlight that future research should be conducted with larger cohorts to increase power. Doing so may increase the sensitivity of analyses such as those based on markers of dietary adherence to detecting further experimental effects. The study groups also had large age ranges, introducing heterogeneity into the data. Age is highly relevant to performance on cognitive tests [36], and although this was controlled for in the analyses, it is possible that this nonetheless impacted analyses to an extent. Future research should use more tightly defined experimental groups. Finally,

a cut-off of 4 weeks post-diagnosis was used for the newly diagnosed CD group criteria. This was to ensure that no participant would have engaged with a GFD for a significant length of time, although some heterogeneity in possible treatment effects may still be present within this timeframe.

5. Conclusions

In summary, in this study, we have demonstrated cognitive deficit in CD revolving around verbal memory, visual memory and visuoconstructive abilities. Stratifying by disease duration, the pattern of these findings suggests that cognitive deficit is established at the point of diagnosis, and though it remains generally stable after this point, some further decline may be expected. QoL was also observed to be impacted in established CD with respect to bodily pain. The extent to which these problems are due to issues around dietary compliance remains an open and important question for further research.

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Current Evidence on the Efficacy of Gluten-Free Diets in Multiple Sclerosis, Psoriasis, Type 1 Diabetes and Autoimmune Thyroid Diseases

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Abstract: In this review, we summarize the clinical data addressing a potential role for gluten in multiple sclerosis (MS), psoriasis, type 1 diabetes (T1D) and autoimmune thyroid diseases (ATDs). Furthermore, data on the prevalence of celiac disease (CD) and gluten-related antibodies in the above patient groups are presented. Adequately powered and properly controlled intervention trials investigating the effects of a gluten-free diet (GFD) in non-celiac patients with MS, psoriasis, T1D or ATDs are lacking. Only one clinical trial has studied the effects of a GFD among patients with MS. The trial found significant results, but it is subject to major methodological limitations. A few publications have found beneficial effects of a GFD in a subgroup of patients with psoriasis that were seropositive for anti-gliadin or deamidated gliadin antibodies, but no effects were seen among seronegative patients. Studies on the role of gluten in T1D are contradictive, however, it seems likely that a GFD may contribute to normalizing metabolic control without affecting levels of islet autoantibodies. Lastly, the effects of a GFD in non-celiac patients with ATDs have not been studied yet, but some publications report that thyroid-related antibodies respond to a GFD in patients with concomitant CD and ATDs. Overall, there is currently not enough evidence to recommend a GFD to non-celiac patients with MS, psoriasis, ATDs or T1D.

Keywords: gluten; gluten-free diet; gliadin; autoimmunity; neurology; multiple sclerosis; psoriasis; autoimmune thyroid disease; type 1 diabetes; celiac disease

1. Introduction

Wheat is a major component of Western diets, however, abstaining from gluten is becoming a popular trend [1]. Adhering to a lifelong gluten-free diet (GFD) is the current treatment for celiac disease (CD)—an immune-mediated small intestinal enteropathy triggered by the ingestion of gluten [2]. It has been hypothesized that gluten may contribute to deteriorating the course of immune-mediated disorders [3–5]. According to a U.S. national survey, a GFD was the most common special diet to be used by patients with psoriasis [6]. Similarly, an American dietary survey found that 5.6% of the surveyed patients with multiple sclerosis (MS) reported adhering to a GFD [7], whereas in an Australian survey, a GFD was adopted by 16.4% of the included patients with MS [8]. Type 1 diabetes (T1D) and autoimmune thyroid diseases (ATDs) affect the endocrine system. The contribution of dietary factors to the pathogenesis of autoimmune endocrine disorders is currently an active research

area. This review summarizes the currently available clinical data on a potential involvement of gluten in MS, psoriasis, T1D and ATDs.

2. Gluten

Gluten proteins have long been of interest to the food industry due to their high impact on the baking quality of wheat flours [9,10]. From a chemical perspective, gluten has been defined as the proteinaceous mass that remains when wheat dough is washed with water and consists primarily of the prolamin and glutelin fractions of the storage proteins of wheat [11,12]. The terms prolamin and glutelin originate from the classification of grain proteins into four fractions according to their solubility properties (Osborne fractions) [13]. Prolamins are insoluble in water but soluble in alcohol, whereas glutelins are insoluble in both water and alcohol [14]. The terms gliadin and glutenin account for the prolamin and glutelin fractions of wheat, whereas the terms secalin, hordein and avenin describe the prolamin fraction of rye, barley and oats, respectively [14]. Likewise, the glutelin fractions of rye and barley are commonly described as secalinin and hordenin, however, similar terminology does not apply for oat glutelins [12]. Codex Alimentarius has defined gluten as "a protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives thereof, to which some persons are intolerant and that is insoluble in water and 0.5M NaCl" [15]. As a result, gluten is nowadays considered to be a common term for the prolamin and glutelin fractions of wheat, rye, barley and, in some cases, oats.

Gluten proteins contain repetitive sequence sections that are rich in the amino acids proline and glutamine [14,16]. Such sections cannot be fully degraded by the human gastrointestinal enzymes [10,13], resulting in the presence of relatively long gluten peptides in the small intestine. In patients with CD, such gluten peptides trigger an inflammatory reaction, however, their presence in the small intestine of most healthy individuals is believed to be rather unproblematic. In vitro studies using caco-2 cell lines [17,18] as well as ex vivo studies on human biopsy explants from both CD patients and healthy controls (HCs) [18,19], suggest that exposure to gliadin disrupts the integrity of the intestinal epithelium. The effect of gliadin on intestinal permeability is believed to be mediated through the secretion of the protein zonulin [20]. Zonulin has been identified as prehaptoglobin-2 [21] and serum zonulin is often used as a marker of intestinal permeability. Levels of zonulin have been found to be elevated in autoimmune diseases [22–25], however, widely used ELISA kits cross-react with proteins, such as properdin and complement C3 [26,27], which shows why caution should be practiced when interpreting data on this topic.

3. Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune, yet incurable, disease of the central nervous system [28] and one of the leading causes of disability among young adults. A recent publication reports that 31% (10/32) of websites providing MS-specific dietary advice recommend patients with MS to abstain from "grains (gluten)" [29]. Despite a high interest in the use of dietary modifications to ameliorate the course of the disease [30], MS-specific, evidence-based dietary guidelines have not been developed yet.

3.1. Gluten-Free Interventions in Multiple Sclerosis

The effects of a GFD among patients with MS have only been investigated by a single open label, non-randomized, controlled trial. Thirty-six patients, who followed a GFD for a median of 4.5 years (mean 5.3 ± 1.6), were compared with 36 patients who followed a regular diet [31]. At the end of the study, the group on the GFD had significantly lower disability measured by the expanded disability status scale (EDSS) (1.5 ± 1.4 vs. 2.1 ± 1.5, $p = 0.001$, baseline EDSS was 1.7 for both groups) and significantly lower activity on magnetic resonance imaging (MRI) (28% vs. 67%, $p = 0.001$) compared to the group on a regular diet [31]. There was no effect on annual relapse rate. Unfortunately, this study was subject to important limitations. Group allocation was performed by instructing all 72 patients to follow a GFD for the first six months of the study, whereafter non-compliant patients were asked to

resume a regular gluten-containing diet. In addition, eight study participants were diagnosed with CD and they all remained in the GFD group, further supporting the idea that the method used for group allocation was suboptimal. Apart from above described methodological issues, major inconsistencies, including the use of the word "randomised" in the title of the study, reduce our capacity to trust this publication [31].

Eliminating gluten from the diet is also part of the "The Wahls Protocol", a multimodal lifestyle intervention including, among others, adherence to a modified paleolithic diet. Clinical studies have illustrated that "The Wahls Protocol" can contribute to improving primarily self-reported outcomes, such as mood, fatigue and quality of life among patients with relapsing remitting MS [32] and progressive MS [33–35]. The risk of placebo and/or nocebo effects should not be neglected when evaluating the results of lifestyle interventions. Furthermore, due to the multimodal nature of the interventions, it is not possible to quantify the effects of eliminating gluten from the diet. The lack of disease-specific endpoints is a major limitation of these studies. Nevertheless, these publications highlight that lifestyle modifications can contribute to improving the quality of life of patients with MS. This is of utmost importance for patients with the progressive forms of MS, as highly effective treatments for these patients are still lacking [36].

3.2. Prevalence of Celiac Disease and Gluten-Related Serology in Multiple Sclerosis

Several publications have reported the prevalence of gluten-related antibodies among patients with MS. Among six studies estimating the prevalence of seropositivity for anti-gliadin (AGA) immunoglobulins (Ig) in patients with MS [37–42], only one study found a significantly higher prevalence of IgG-AGA among patients with MS (7/98) compared to HCs (2/140) ($p = 0.03$) [42]. However, when investigating whether patients with MS have elevated mean values of IgA-AGA or IgG-AGA compared to HCs, the results are highly contradictive [38,42–44]. We can therefore not exclude that patients with MS may have slightly elevated AGA titers compared to HCs, however, this is still far from sufficiently different for diagnostic use.

Data from twelve studies [37–48] estimating the prevalence of seropositivity for IgA tissue transglutaminase (tTG) in patients with MS do not support an increased prevalence of CD among patients with MS, whereas a single study found higher mean values of IgA-tTG and IgG-tTG among 30 patients with MS compared to 25 HCs [49]. So far, only one publication supports an association between CD and MS by reporting the prevalence of CD to be 11% in a cohort of 72 patients with relapsing remitting MS and 32% (23/126) among their first-degree relatives [50]. According to the last-mentioned study, the diagnosis of MS was made at a younger age among celiac (35 +/− 7 years) compared to non-celiac (44 +/− 10 years) patients ($p < 0.05$) [50]. For an overview of studies measuring other gluten- and celiac-related antibodies among patients with MS, the reader is referred to Thomsen et al. (2019) [51].

The most powerful studies investigating a potential association between CD and MS are two Danish population-based studies [52,53] and a Swedish case–control study including 14,371 CD patients and 70,096 reference individuals [54], however, none of them found any association. The first Danish study investigated the comorbidity of 31 autoimmune diseases and calculated an odds ratio (OR) of 1.0 for CD and MS [52]. The second Danish study investigated the prevalence of autoimmune comorbidities among patients with CD and failed to find an increased prevalence of MS among patients with CD. According to the Swedish case–control study, the presence of CD did not increase the risk of subsequent MS diagnosis (hazard ratio (HR) = 0.9; 95% confidence interval (CI) = (0.3–2.3)) [54]. Lastly, two French studies estimated the prevalence of MS among patients with CD to be 0.11% (1/924) [55] and 0.14% (1/741) [56]. This is similar to the crude estimate of MS in the French population, which is 0.15% [57].

4. Psoriasis

Psoriasis is a chronic autoimmune skin disease characterized by the development of erythematous scaly lesions. Psoriasis vulgaris, also known as plaque psoriasis, is the most common type of psoriasis, but several other types of psoriasis also exist [58]. The Medical Board of the National Psoriasis Foundation conducted a systematic review in 2018 with the aim of developing nutritional recommendations for patients with psoriasis or psoriatic arthritis [59]. The board states "We weakly recommend a gluten-free diet only in patients who test positive for serologic markers of gluten sensitivity" [59]. The popularity of the GFD among patients with psoriasis is highlighted in a U.S. study from 2017, in which 38% of the responding patients (*n* = 1206) reported avoiding gluten and 53.4% (247/459) of them reported to have experienced an improvement or clearance of their disease as a result of the GFD [6].

4.1. Intake of Gluten and Risk of Psoriasis

Using data from the Nurses´ Health Study II, a publication examined whether higher intakes of gluten were associated with a higher risk of future psoriasis, psoriatic arthritis and atopic dermatitis [60]. When comparing the highest and lowest gluten intake quintiles, the multivariate HRs were 1.15 (95%CI = (0.98–1.36)), 1.12 (95%CI = (0.78–1.62)) and 0.91 (95%CI = (0.66–1.25)) for psoriasis, psoriatic arthritis and atopic dermatitis, respectively. No dose–response relationship was observed, but the fact that the effect of a strictly GFD was not investigated is a minor limitation of this study.

4.2. Gluten-Free Interventions in Psoriasis

The potential role of gluten in psoriasis has been addressed in several publications from Michaëlsson and his colleagues. In 2000, they published a study illustrating clinical improvement in 73% (22/30) of patients who adhered to a GFD for three months (reduction of psoriasis area and severity index (PASI) score from 5.5 ± 4.5 to 3.6 ± 3.0 (*p* = 0.001)) [61]. All patients were positive for IgA-AGA or IgG-AGA and no clinical improvement was observed among six seronegative patients who also adhered to a GFD. The study was originally designed as a cross-over trial and, after three months on a GFD, the participants had to reintroduce gluten to their diet for three months. However, the last part of the study was discontinued as 60% (18/30) of the AGA-positive patients, but none of the seronegative patients, required increased treatment due to a worsening of their skin lesions after the reintroduction of dietary gluten [61]. Immunohistochemical analyses of skin biopsies from 19 of the above seropositive patients were later published in a separate publication that revealed a reduction in Ki67 positive cells in the involved dermis after the GFD [62]. Moreover, a higher expression of tTG was found in involved, compared to uninvolved, dermis (5.06 ± 3.80% vs. 0.67 ± 0.54%, *n* = 13, *p* = 0.0002) and the GFD resulted in a drop in tTG expression in the dermis by 50% [62].

Similarly, in 2007 Michaëlsson et al. presented results from 16 cases of palmoplantar pustulosis, who adhered to a GFD [63]. AGA-seropositive patients who strictly adhered to the GFD (*n* = 9) experienced great improvements or even the clearance of their lesions. Improvements were only seen in two out of four patients with lower compliance to the GFD and none of the seronegative patients (*n* = 3) [63].

According to a more recent publication by Kolchak et al. in 2018, a one-year gluten-free intervention resulted in a 56% and 36% improvement in the PASI score in patients with very high (>30 U/ml, *n* = 5) and high (11.5–30.0 U/ml, *n* = 8) levels of IgA against gliadin peptides (not clear whether native or deamidated gliadin), respectively [64]. As no other antibodies were measured and biopsies were not performed, it is not known whether some of the included patients suffered from CD. The effects of a GFD in patients with concomitant psoriasis and CD have been explored in an Italian multicenter study [65]. At a three-month follow-up, patients (*n* = 9) experienced major improvements in their PASI scores (two by at least 50%, five by at least 75%, total clearance in one patient and one drop-out). A single patient had worsened by the six-month follow-up, whereas most patients maintained their

clinical improvement (*n* = 5) and two patients further improved [65]. Overall, evidence suggests that psoriasis patients with gluten-related antibodies may benefit from a GFD, however, larger trials are still lacking.

4.3. Gluten-Related Serology in Psoriasis

According to a meta-analysis from 2014, both the prevalence of seropositivity for IgA-AGA, as well as mean values of IgA-AGA, are higher among patients with psoriasis compared to HCs [66]. To illustrate the heterogeneity among studies, an overview of identified case–control publications is provided in Table 1. Additional studies have described the prevalence of AGA seropositivity among patients with psoriasis [67,68] and palmoplantar pustulosis [63] without including a relevant control group. Moreover, most studies [69–72] have found significantly higher concentrations of IgA-AGA among patients with psoriasis compared to HCs, whereas one study [73] did not. Regarding IgG-AGA, only one [71] out of four studies [70,72,73] found higher concentrations in patients with psoriasis compared to HCs. Using a different approach, no difference was found in the proliferative response of peripheral blood mononuclear cells from patients with psoriasis (*n* = 37) and HCs (*n* = 37) after stimulation with wheat peptides, however, the five highest responses against peptide p62-75 were observed among patients with psoriasis [74].

Table 1. Case–control studies estimating the prevalence of IgA-AGA and IgG-AGA in patients with psoriasis and HCs. Results are presented as "number of seropositive individuals"/"number of individuals tested". HCs: healthy controls, Ig: immunoglobulin, AGA: antigliadin antibody, NA: not available, NS: not significant.

Case–Control Studies	IgA-AGA in Psoriasis	IgA-AGA in HCs	*p*-Value	IgG-AGA in Psoriasis	IgG-AGA in HCs	*p*-Value
Akbulut 2013 [75]	6/37	1/50	*p* = 0.039	3/37	0/50	*p* = 0.073
Cardinali 2002 [76]	0/39	0/39	NS	2/39	0/39	NA
Kalayciyan 2006 [77]	21/127	3/31	NS	-	-	-
Kia 2007 [78]	6/200	5/100	NS	32/200	16/100	NS
[1] Kolchak 2018 [64]	13/97	2/91	NA	-	-	-
Lesiak 2016 [79]	0/20	0/29	NS	-	-	-
Nagui 2010 [69]	14/41	1/41	NA	-	-	-
Singh 2010 [71]	8/56	0/60	*p* < 0.05	12/56	0/60	*p* < 0.05
Sultan 2010 [73]	8/120	9/120	NS	5/120	6/120	NS
In total	76/737	21/561	-	54/452	22/369	-

[1] Possibly measures of antibodies against deamidated gliadin peptide.

To investigate whether gluten-related antibodies correlate with disease activity in psoriasis, a study screened 130 patients for IgG-AGA, IgA-AGA and IgA-tTG and identified 21 patients (16.2%) who were positive for at least one of the antibodies [80]. Psoralen and ultraviolet A (PUVA) phototherapy (57% vs. 30%, *p* = 0.03) and systemic therapy (48% vs. 22%, *p* = 0.04) was currently given or had previously been given to a higher percentage of seropositive compared to seronegative patients. There was no difference for ultraviolet B (UVB) phototherapy and the presence of arthritis or arthralgia [80]. A similar but smaller study (*n* = 41) found a significant relationship between seropositivity for IgA-AGA and disease duration (*p* < 0.001), however, being seropositive was not related to PASI scores [69]. In contrast, in a study with 120 patients with psoriasis (eight seropositive for IgA-AGA/five seropositive for IgG-AGA), severe disease at the reported time or past treatment for high disease severity was not associated with seropositivity for AGA [73].

According to a publication from 2020 that tested for antibodies against an array of 75 antigens, IgG4 antigliadin antibodies were the only antibodies to be elevated in the sera of 12 patients with severe psoriasis (PASI > 30) [81]. IgG4 antigliadin antibodies were not present in sera from 12 HCs. Later validation, using a cohort with 73 psoriasis patients and 75 HCs, resulted in an area under the curve of 0.98 ($p < 0.001$) in the receiver operating characteristic (ROC) analysis, suggesting that IgG4 antigliadin antibodies could potentially function as a diagnostic biomarker for psoriasis. For a subgroup of patients with the highest levels of anti-gliadin IgG4, there was a significant correlation between antibody levels and PASI scores (r = 0.65, $p < 0.001$) [81].

With regard to the prevalence of IgA-tTG antibodies in psoriasis, we have identified five cross-sectional cohort studies [63,68,80,82,83] and ten case–control studies [65,69,71,75,76,84–88] (previously mentioned in Table 2). In addition, a study of 67 patients with psoriasis and 85 HCs found significantly elevated mean values of IgA-tTG in patients with psoriasis (0.943 ± 1.131 vs. 0.852 ± 0.576, $p < 0.05$) [71]. Many case–control studies fail to reveal a significant difference between groups, however, it must be stressed that the majority of studies on the topic are underpowered, considering the fact that the global seroprevalence of CD has been estimated to be 1.4% [89].

Table 2. Case–control studies estimating the prevalence of IgA-tTG in patients with psoriasis and HCs. Results are presented as "number of seropositive individuals"/"number of individuals tested". HCs: healthy controls, IgA-tTG: class A immunoglobulins against tissue transglutaminase, NA: not available, NS: not significant.

Case–Control Studies	IgA-tTG in Psoriasis	IgA-tTG in HCs	*p*-Value
Akbulut 2013 [75]	1/37	0/50	NS
Bastiani 2015 [65]	9/218	1/264	$p < 0.05$
Cardinali 2002 [76]	1/39	0/39	NS
Hull 2008 [84]	0/37	1/53	NS
Juzlova 2016 [85]	2/189	0/378	$p = 0.045$
Montesu 2011 [86]	2/100	0/100	NS
Nagui 2010 [69]	14/41	9/41	NA
Ojetti 2003 [87]	2/92	0/90	NA
Riente 2004 [88]	1/75	3/78	NS
Singh 2010 [71]	6/56	0/60	$p = 0.01$
In total	38/884	14/1153	-

4.4. Comorbidity between Celiac Disease and Psoriasis

Results from 18 publications included in a systematic review and meta-analysis from 2019 are summarized below [90]. Out of two studies investigating the incidence of CD among patients with psoriasis, only one found a statistically significant increased risk (HR = 1.9, 95%CI = (1.6–2.2) [91] and HR = 1.20, 95%CI = (0.91–1.59)) [92]. Similarly, two studies estimated the incidence of psoriasis among patients with CD, but in this case, both studies found significant results (HR = 1.72, 95%CI = (1.54–1.92) [93] and HR = 1.9, 95%CI = (1.5–2.3) [91]). With regard to the prevalence of CD in patients with psoriasis or psoriatic arthritis, significantly increased ORs were found in five [65,92,94–96] out of nine [86,87,97,98] studies (meta-analysis: OR = 2.16, 95%CI = (1.74–2.69) [90]). Likewise, the prevalence of psoriasis among patients with CD was found to be increased in four [53,93,99,100] out of eight [101–104] publications (meta-analysis: OR = 1.8, 95%CI = (1.36–2.38) [90]). The two-way meta-analysis concluded that clinicians should be aware of the significant association between CD and psoriasis [90].

5. Type 1 Diabetes

T1D is a chronic, autoimmune disease characterized by the destruction of the insulin-producing beta cells in the pancreas. T1D is often diagnosed in childhood and results in a lifelong need for exogenous insulin. Several animal studies support the potential involvement of gluten in the

pathogenesis of T1D [105] and have previously been summarized by Antvorskov et al. [105] and Haupt-Jorgensen et al. [106].

5.1. Exposure to Gluten during Early Life and Risk of Type 1 Diabetes

Recent mother and child cohort studies suggest that exposure to gluten during early life may affect the risk of developing T1D [107,108], whereas earlier studies among predisposed individuals did not reveal such an association [109,110]. According to a Danish study from 2018 [107], offspring from mothers with the highest intake of gluten had a twofold higher risk of developing T1D compared to offspring from mothers with the lowest intake of gluten during pregnancy (adjusted HR = 2.00, 95%CI = (1.02–4.00)). A dose–response relationship was demonstrated, however, only the difference between the groups with the highest and lowest intakes of gluten reached statistical significance. These results were not replicated in a similar Norwegian study from 2020 [108] but, this time, the intake of gluten by the offspring themselves was associated with a higher risk of T1D (adjusted HR = 1.46, 95%CI = (1.06–2.01), p = 0.02).

Similarly, publications have explored whether and how infant feeding patterns could affect the risk of T1D [105]. Data from the Diabetes Autoimmunity Study in the Young (DAISY) support that introduction of cereals between the age of 4-6 months leads to the lowest risk of islet autoimmunity (<4 months: HR = 4.32, 95%CI = (2.0–9.35), >6 months: HR = 5.36, 95%CI = (2.08–13.8)) [111]. Likewise, the late (≥7 months) introduction of gluten-containing porridge has been found to be a risk factor for the development of β-cell autoantibodies [112], whereas Ludvigsson [113] did not find an association between the time of the introduction of gluten and levels of islet autoantibodies. Results from the BABYDIAB study support the idea that the introduction of gluten-containing foods at or before the age of three months increases the risk of islet autoimmunity (HR = 5.2, 95%CI = (1.7–15.5), p = 0.003), however, the late (>6 months) introduction of gluten-containing foods was not associated with increased risk of islet autoimmunity [114]. Lastly, the BABYDIET study—a pilot study in which 150 infants at high risk of T1D where randomized to either control (6 months) or late (12 months) introduction of gluten—did not find any difference in islet autoimmunity at three years in the per protocol analysis (compliance = 70%) [115].

5.2. Gluten-Free Interventions in Type 1 Diabetes

Two studies have investigated whether a GFD could have a protective effect among children with a high risk of developing T1D. In the first study, 17 first-degree relatives of T1D patients with at least two β-cell autoantibodies were included in a cross-over trial consisting of six months on a GFD followed by six months on a gluten-containing diet [116]. Glucose tolerance tests revealed an improved acute insulin response after the GFD (p = 0.004) and a non-significant deterioration after the reintroduction of dietary gluten (p = 0.07). The results were similar for insulin sensitivity measured by the homeostasis model of insulin resistance (HOMA-IR), however, this time, a non-significant increase after the GFD was followed by a significant decrease (p < 0.005) after the gluten-containing diet. An effect on the levels of autoantibodies was neither observed in this study [116] nor in a similar study with a longer gluten-free intervention of 12 months (n = 7) [117]. A five-year follow-up to the latter study suggests that the 12 months on a GFD did not affect the risk of progressing to T1D [117].

In 2012, a case report suggested that a GFD introduced 2–3 weeks after the diagnosis of T1D may have prolonged remission in a five-year-old boy without CD [118]. Both his HbA1c and his fasting blood glucose stabilized without insulin therapy and, twenty months after diagnosis, he still remained without the need for daily insulin therapy [118]. This case led to the performance of a Danish pilot study evaluating the effects of a one-year gluten-free intervention among 15 children with newly diagnosed T1D [119]. Compared to two previous reference cohorts, the children on the GFD had a 21% lower HbA1c and a higher prevalence of partial remission (insulin dose-adjusted A1c (IDAA1c) ≤ 9), but no difference was seen in stimulated C peptide [119].

Prolonged partial remission in response to a GFD was also illustrated in a study that was methodologically stronger due to the inclusion of a control group which remained on a standard gluten-containing diet (*n* = 19) during the study time [120]. The trial was not randomized and 20 out of 26 children completed the one-year gluten-free intervention with satisfactory compliance. The GFD was introduced within a median of 38 days from the onset of T1D. At follow-up, the children adhering to the GFD had a lower IDAA1c (by 1.37; *p* = 0.01), a lower mean HbA1c (by 0.7% (7.8 mmol/mol); *p* = 0.02) and there was a tendency towards a lower insulin dose (by 0.15 U/kg/day; *p* = 0.07) compared to the control group [120]. Last, but not least, several studies have investigated whether a GFD affects metabolic control in individuals with both CD and T1D [121–142] (presented in Table A1 in Appendix A).

5.3. Prevalence of Celiac Disease and Gluten-Related Serology in Type 1 Diabetes

A systematic review and meta-analysis published in 2014 calculated the prevalence of biopsy-confirmed CD among patients with T1D to be 6.0% (95%CI = (5.0–6.9%)) [143]. Similarly, a systematic review and meta-analysis from 2019 reports the weighted prevalence of CD and any gluten-related antibodies among patients with T1D to be 4.7% (95%CI = (4.0–5.5)) and 10.2% (95%CI = (8.4–12.7)), respectively [144]. Among gluten-related antibodies, the highest weighted prevalence was estimated for IgG-AGA as 12.7% (95%CI = (6.1–21.0)) [144]. Equally relevant, a Swedish population cohort study has estimated the HR of subsequent T1D before the age of 20 years to be 2.4 (95%CI = (1.9–3.0), *p* < 0.001) among patients with CD [145].

Although the association between CD and T1D is well supported, the heterogeneity among studies is large. A better understanding of the factors that contribute to this variation may therefore be relevant. With regard to measurements of gluten-related antibodies, technical differences in the analytical assays being used may hinder direct comparisons among publications highlighting the importance of including a healthy control group in all studies. This is especially relevant for AGA due to their lower specificity for CD and the fact that biological factors may contribute to their variation within healthy populations.

The meta-analysis by Elfström et al. [143] revealed that CD was less frequent in adults (2.7%, 95%CI = (2.1–3.3%)) compared to children (6.2%, 95%CI = (6.1–6.3%)) with T1D (*p* < 0.001). Tiberti et al. [146] on the contrary, found a significantly higher prevalence of gluten-related antibodies among patients with a high (> 15 years) compared to a low (5–15 years) duration of T1D. Similarly, Nederstigt et al. [144] reported that the prevalence of IgA-AGA increased with the duration of T1D, whereas endomysium antibodies decreased with age. Interestingly, IgA-tTG-seropositive patients with T1D have been found to have lower titers of IgG-tTG and deamidated gliadin peptide antibodies compared to CD patients without T1D [147]. In addition, longitudinal studies suggest that AGA titers can fluctuate over time [148] but also that diabetes-related antibodies may respond to a GFD in cases with CD [149]. Lastly, data from Salardi et al. [150] support that the prevalence of CD significantly increased among Italian patients with T1D after 1994, however, this might also reflect an increase in the prevalence of CD in the general population.

6. Autoimmune Thyroid Diseases

ATDs affect 2–5% of the population with a female predominance. The most common ATDs are Hashimoto's thyroiditis (HT) and Graves' disease, which lead to hypothyroidism and hyperthyroidism, respectively [114].

6.1. Gluten-Free Interventions in Autoimmune Thyroid Diseases

Few studies have investigated whether a GFD can contribute to ameliorating thyroid-related pathology among patients with concomitant CD, but we have not been able to identify publications exploring the effects of a GFD in ATDs in the absence of CD or celiac-related antibodies.

A controlled trial has investigated the effects of six months on a GFD (*n* = 16) compared to no dietary intervention (*n* = 18) among drug-naive women with HT [151]. The GFD resulted in a drop in the levels of thyroid peroxidase (TPO) and thyroglobulin antibodies, an increase in 25-hydroxyvitamin D and an improvement in the structure parameter inference approach (SPINA)-GT index, which correlated with the changes in antibody titers. No effect was seen on levels of thyrotropin and free triiodothyronine. The study population included patients that were seropositive for IgA-tTG, however, no intestinal biopsies were performed and patients with symptomatic CD were excluded [151].

An Italian multicenter study evaluating the thyroid function of 128 patients with newly diagnosed CD before and one year after the introduction of a GFD reports that, in some patients, a GFD can reverse thyroid abnormalities [152]. Valentino et al. [153] also noted an improvement in symptoms related to hypothyroidism and thyroxine dosage among three ATD patients with concomitant CD that followed a GFD for six months. However, levels of thyroglobulin and TPO antibodies only changed for one patient, who had an additional follow-up at 18 months [153].

On the contrary, Mainardi et al. [154] report that a GFD did not seem to influence thyroid function and antibodies among two cases of concomitant CD and ATD. Likewise, a more recent study found no effect of one year on a GFD on levels of TPO antibodies that were present among 10 (37%) patients with newly diagnosed CD [155]. On the contrary, thyroid volume significantly decreased compared to a group of patients without CD, indicating that thyroiditis was continually progressing even after the establishment of a GFD [155]. It is possible that a longer study time is necessary to reveal an effect of a GFD, as TPO antibodies were only present among 76.9% (10/13), 46.1% (6/13) and 15.3% (2/13) of CD patients with ATD at, respectively, 6-, 12- and 24-month follow-ups on a GFD [149].

Interestingly, a study found that patients with concomitant CD and HT (*n* = 14) needed an almost 50% higher dose of levothyroxine to reach target thyroid-stimulating hormone (TSH) values when compared to patients with HT alone (*n* = 68) [156]. The authors suggest that this could potentially be explained by reduced absorption of levothyroxine in cases of untreated CD, as an increased need for levothyroxine was prevented by the introduction of a GFD (*n* = 21). However, reduced absorption capacity cannot explain why patients with concomitant HT and CD had significantly higher TSH (5.7 vs. 7.26, *p* = 0.0099) and significantly lower free T4 (1.12 vs. 0.01, *p* < 0.0001) compared to patients with isolated HT before the initiation of levothyroxine treatment [156]. In accordance with the above, Zubarik et al. [157] reported that patients requiring high doses of levothyroxine to maintain an euthyroid state were more likely to have CD, but this was not confirmed by Sharma et al. [158].

6.2. Gluten-Related Serology in Autoimmune Thyroid Diseases

Identified studies measuring levels of IgA-AGA and IgG-AGA in ATDs are summarized in Table 3. Furthermore, a study measuring the presence of IgG antibodies against 125 foods found no difference in IgG positivity for wheat or gliadin between 74 patients with HT and 245 HCs [180], but IgG positivity for barley was significantly higher among patients with HT compared to HCs (93.2% vs. 71.0%, *p* = 8.4×10^{-5}) [180]. Moreover, a study supporting the previously discussed association between CD and an increased need for levothyroxine found that patients treated with high dosage of levothyroxine (125–200 µg/day) had significantly higher levels of IgA-AGA compared to patients receiving low levels of levothyroxine (50–100 µg/day) (medians: 19.69 vs. 13.00, *p* = 0.033) [162].

Table 3. Case–control and cross-sectional cohort studies estimating the prevalence of IgA-AGA and IgG-AGA in patients with ATD and HCs. Results are presented as "number of seropositive individuals"/"number of individuals tested" (%). AGA: antigliadin antibody, ATD: autoimmune thyroid disease, HCs: healthy controls, Ig: immunoglobulin, NA: not available.

Study	IgA-AGA in ATD	IgA-AGA in HCs	p-Value	IgG-AGA in ATD	IgG-AGA in HCs	p-Value	IgA-tTG in ATD	IgA-tTG in HCs	p-Value
Ch'ng 2005 [159]	15/111 (13.5%)	-	-	-	-	-	2/111 (1.8%)	1/115 (0.9%)	NA
Guliter 2007 [160]	-	-	-	-	-	-	8/136 (5.8%)	1/119 (0.8%)	p = 0.04
Hadithi 2007 [161]	9/104 (8.7%)	-	-	7/104 (6.7%)	-	-	8/104 (7.7%)	-	-
Jiskra 2003 [162]	27/169 (16.0%)	101/1312 (7.7%)	p = 0.002	87/169 (51.5%)	92/1312 (7.0%)	p < 0.001	25/169 (14.8%)	-	-
Mainardi 2002 [154]	-	-	-	-	-	-	2/100 (2%)	-	-
Mankai 2006 [163]	-	-	-	-	-	-	6/161 (3.7%)	-	-
Marwaha 2013 [164]	-	-	-	-	-	-	40/577 (6.9%)	20/577 (3.5%)	p = 0.015
Mehrdad 2012 [165]	3/454 (0.7%)	-	-	18/297 (6.1%)	-	-	8/454 (1.8%)	-	-
Meloni 2000 [166]	13/297 (4.4%)	-	-	46/737 (6.2%)	-	-	-	-	-
Ravaglia 2003 [167]	-	-	-	-	7/600 (1.2%)	NA	11/737 (1.5%)	2/600 (0.3%)	p = 0.046
Riseh 2017 [168]	6/40 (15.0%)	5/42 (11.9%)	NA	2/40 (5.0%)	4/42 (9.5%)	NA	9/40 (22.5%)	7/42 (16.6%)	NS
Sahin 2018 [169]	-	-	-	-	-	-	3/66 (4.6%)	-	-
Sari 2009 [170]	-	-	-	-	-	-	8/101 (7.9%)	0/103 (0.0%)	NA
Sattar 2011 [171]	-	-	-	-	-	-	14/302 (4.6%)	-	-
Sharma 2016 [158]	-	-	-	-	-	-	24/280 (8.6%)	-	-
Spadaccino 2008 [172]	-	-	-	-	-	-	10/271 (3.7%)	-	-
Tuhan 2016 [173]	-	-	-	-	-	-	1/80 (1.3%)	-	-
Twito 2018 [174]	-	-	-	-	-	-	5/114 (4.4 %)	-	-
Valentino 2002 [175]	0/14 (0.0%)	-	-	0/14 (0.0%)	-	-	0/14 (0.0%)	-	-
Ventura 2014 [176]	-	-	-	-	-	-	2/53 (3.8%)	-	-
Volta 2001 [177]	-	-	-	6/220 (2.7%)	3/250 (1.2%)	NA	7/20 (3.2%)	1/250 (0.4%)	p = 0.022
Zhao 2016 [178]	-	-	-	-	-	-	26/119 (21.9%)	1/102 (1.0%)	p < 0.0001
Zubarik 2015 [179]	-	-	-	-	-	-	10/499 (2.0%)	-	-

An interesting study found that the prevalence of chronic thyroiditis or seropositivity for TPO antibodies was higher among 16 patients with T1D that were seropositive for AGA compared to 37 AGA-seronegative T1D patients (38% vs. 2.7%, $p = 0.005$ for chronic thyroiditis and 69% vs. 27%, $p = 0.01$ for TPO seropositivity) [181]. This is further supported by a study reporting that the prevalence of tTG ($p = 0.023$) and glutamic acid decarboxylase (GAD) ($p < 0.00001$) antibodies increased with increasing titers of TPO antibodies [164]. A correlation between TPO and IgA-tTG antibodies has also been illustrated in a study suggesting that IgA-tTG may contribute to thyroid dysfunction by binding to thyroid tissue [182]. Similarly, IgG-tTG and IgA-AGA have been found to be predictors of TPO and thyroglobulin antibodies, respectively (IgG-tTG/ TPO: β = 0.12, 95%CI = (0.03–0.21), $p = 0.008$, IgA-AGA/thyroglobulin: β = −0.10, 95%CI = (−0.19–−0.002), $p = 0.045$) [168]. The association between ATDs, T1D and CD has been confirmed by additional publications [178,183], including a population-based cohort study concluding that CD is a risk factor for later development of ATDs among patients with T1D [184].

6.3. Comorbidity between Celiac Disease and Autoimmune Thyroid Diseases

A systematic review and meta-analysis of 27 studies calculated the median prevalence of CD in ATDs to be 3.2%, however, a pooled analysis resulted in a prevalence of 1.6% (CI = (1.3–1.9%)) for biopsy-verified CD [185]. The abovementioned low prevalence can possibly be explained by the fact that intestinal biopsies are not performed in all seropositive patients with potential CD. Furthermore, the prevalence of CD was higher among children with ATDs (6.2%, CI = (4.0–8.4%)) compared to adults (2.7%, CI = (2.1–3.4)) [185], whereas another study suggests that the prevalence of CD is higher among patients with ATDs above the age of 65 [167].

A meta-analysis of a systematic review from 2016 revealed a significantly higher prevalence of thyroid disease among patients with CD compared to controls (OR = 3.08, 95%CI = (2.76–3.56)) [186]. Similar results were also found for euthyroid ATD (OR = 4.35, 95%CI = (2.88–6.56)) and hypothyroidism (OR = 3.38, 95%CI = (2.73–4.19)), however, the prevalence of hyperthyroidism among patients with CD did not differ from that in controls (OR = 1.28, 95%CI = (0.37–4.46)) [186]. On the contrary, data from 3209 patients with Grave's disease and 1069 HCs support the idea that the prevalence of CD is higher among patients with Grave's disease (1.1%) compared to HCs (0.3%) (OR = 3.81, 95%CI = (1.17–12.41)) [187]. Additionally, a meta-analysis reports that the prevalence of biopsy-proven CD is higher among patients with hyperthyroidism (2.6%, CI = (0.7–4.4%)) compared to patients with hypothyroidism (1.4%, CI = (1.0–1.9%)) [185]. We hypothesize that the late age of disease onset for hyperthyroidism could be a potential explanation for the above contradictive results. An association between thyroid disease and CD has also been confirmed by more recent studies [188,189]. One calculated that the prevalence of thyroid disease was fourfold higher among 288 patients with untreated CD compared to 250 controls without CD (13.6% vs. 3.2%, $p < 0.05$) [188] and the other calculated the hazard ratio of subsequent hypothyroidism among patients with CD to be 4.64 (95%CI = (2.88–7.46)) [189].

It has been debated whether the late diagnosis of CD and, as a result, the late introduction of a GFD can increase the risk of developing other autoimmune diseases [55,102,103,190]. When a meta-analysis compared treated and untreated patients with CD, no difference was found in the frequency of thyroid disease (OR = 1.08, 95%CI = (0.61–1.92)) [186]. In addition, a study highlights that first-degree relatives of patients with CD also have an increased risk of ATDs [191]. Interestingly, a study reports that the prevalence of ATDs among Irish women with CD has decreased significantly over recent decades [192], whereas another study suggests that the prevalence of autoimmune thyroiditis may be higher among seronegative (26.9%) compared to seropositive (9.7%) patients with CD ($p = 0.002$) [193]. Last, but not least, the prevalence of ATDs has also been reported to be high among patients with non-celiac gluten/wheat sensitivity [194,195] and dermatitis herpetiformis [196].

7. Conclusions

The current level of evidence is yet not sufficient to recommend a GFD to patients with MS, psoriasis, T1D or ATDs. Larger epidemiological studies and meta-analyses of systematic reviews support that psoriasis, T1D and ATDs are all associated with CD, but this does not seem to be the case for MS. The only clinical trial to have studied the effects of a GFD among patients with MS found positive results on important MS-specific outcomes, however, the publication was subject to major limitations. Further studies are warranted to replicate the results found by Rodrigo et al. [31] and clarify whether any beneficial effects could be restricted to specific subgroups of patients. With regard to psoriasis, the currently available data suggest that patients with gluten-related antibodies or CD may benefit from a GFD, however, larger trials are still missing. The majority of studies failed to reveal an effect of a GFD on diabetes-related autoantibodies, however, it seems likely that a GFD may contribute to normalizing metabolic control in patients with T1D. In addition, some publications report that untreated CD can affect metabolic control and diabetic complications in patients with T1D. On the contrary, studies support the idea that thyroid-related antibodies may respond to a GFD in patients with concomitant CD and ATD, however, no studies have addressed the effects of a GFD among non-celiac patients with ATDs to date. Lastly, in patients with concomitant CD and ATD, a GFD may improve the absorption of levothyroxine.

Appendix A

Table A1. Metabolic control and effects of a GFD in patients with concomitant T1D and CD. CD: celiac Disease, GFD: gluten-free diet, NS: not significant, T1D: type 1 diabetes.

Study	Sample Size	Study Design	HbA1c	Insulin Dose	Diabetic Complications	Hypoglycemic Episodes
Abid 2011 [121]	22 CD and T1D	before vs. after 1 year on GFD	NS	before vs. after GFD: 0.88 vs. 1.1, $p < 0.005$	-	before vs. after GFD: Eight (36%) vs. two (9%), $p < 0.07$
Acerini 1998 [122]	Seven CD and T1D	before vs. after 2 years on GFD	NS trend	NS	-	-
Amin 2002 [123]	11 CD and T1D vs. 22 with T1D only	before vs. after 1 year on GFD and comparison with patients with T1D only	CD and T1D vs. T1D only: 8.9 ± 0.3 vs. 9.8 ± 0.3, $p = 0.002$; before vs. after GFD: 8.9 ± 0.1 vs. 8.3 ± 0.1, $p = 0.002$; CD and T1D after GFD vs. T1D only: 8.3 ± 0.2 vs. $10.0 \pm 0.2\%$, $p = 0.02$	NS between groups; increased in both groups at follow-up	-	-
Bakker 2013 [124]	31 CD and T1D vs. 46 with T1D only	before vs. after GFD and comparison with patients with T1D only	NS (CD and T1D vs. T1D only and before vs. after GFD)	NS (CD and T1D vs. T1D only)	protective role of concurrent (treated) CD against retinopathy	-
Fröhlich-Reiterer 2011 [125]	411 CD and T1D vs. 17661 with T1D only	CD and T1D vs. T1D only	NS	NS	NS	NS
Goh 2010 [126]	29 CD and T1D vs. 58 with T1D only	evaluation every 6 months from 1 year prior to CD diagnosis to 1 year after	NS, similar between groups throughout the study	-	-	-
Hansen 2006 [127]	31 CD and T1D	before vs. after 2 years on GFD	NS	-	-	-
Kaspers 2004 [128]	127 with CD and T1D vs. 19796 with T1D only	CD and T1D vs. T1D only	CD and T1D vs. T1D only: $8.1 \pm 1.8\%$ vs. $8.8 \pm 2.4\%$, $p < 0.001$	NS	-	NS

Table A1. *Cont.*

Study	Sample Size	Study Design	HbA1c	Insulin Dose	Diabetic Complications	Hypoglycemic Episodes
Kaur 2020 [129]	30 CD and T1D	prospective randomized controlled trial (1 year on GFD vs. normal diet)	NS between groups, lower after GFD (within group, $p < 0.05$)	NS	-	NS between groups, lower after GFD (within group, $p = 0.03$)
Kaukinen 1999 [130]	22 CD and T1D vs. 22 with T1D only	retrospective and prospective study	NS	NS	-	-
Leeds 2011 [131]	41 CD and T1D vs. 41 with T1D only	before vs. after 1 year on GFD and comparison with patients with T1D only	CD and T1D vs. T1D only: 8.2 vs. 7.5%, $p = 0.05$; before vs. after GFD: improved among compliant patients ($n = 9$, NS)	NS	CD and T1D vs. T1D only: retinopathy: 58.3 vs. 25%, $p = 0.02$; nephropathy: 41.6 vs. 4.2%, $p = 0.009$; peripheral neuropathy: 41.6 vs. 16.6%, $p = 0.11$; NS reduction in advanced nephropathy after GFD	-
Mohn 2001 [132]	18 CD and T1D vs. 26 with T1D only	evaluation every 6 months from 18 months prior to CD diagnosis to 18 months after	NS	NS prior to CD diagnosis; at CD diagnosis: CD and T1D vs. T1D only: 0.6 ± 0.2 vs. 0.9 ± 0.3, $p = 0.05$; increased after GFD	-	CD and T1D vs. T1D only at +/−6 months from CD diagnosis: 4.5 ± 4 vs. 2.0 ± 2.2, $p = 0.01$
Narula 2009 [133]	22 CD and T1D vs. 50 with T1D only	before vs. after 1 year on GFD for eight compliant patients and comparison with patients with T1D only	-	NS increase after GFD; NS difference in change of insulin requirement	-	-
Pham-Short 2013 [134]	129 CD and T1D vs. 2510 with T1D only	CD and T1D vs. T1D only; compliant vs. non-compliant to GFD	CD and T1D vs. T1D only: 8.3 (7.6–9.3) vs. 8.6 (7.7–9.6), $p = 0.04$; compliant vs. non-compliant: 8.2 (7.6–9.0) vs. 8.7 (7.8–10.0), $p = 0.003$	CD and T1D vs. T1D only: 1.08 (0.91–1.34) vs. 1.05 (0.87–1.28), $p = 0.08$; compliant vs. non-compliant: 1.03 (0.88–1.27) vs. 1.15 (0.99–1.46), $p = 0.002$	elevated albumin excretion rate: compliant vs. non-compliant: 23 vs. 40%, $p = 0.04$; CD and T1D vs. T1D only: NS; retinopathy, peripheral nerve and pupillary abnormality: NS	-

Table A1. *Cont.*

Study	Sample Size	Study Design	HbA1c	Insulin Dose	Diabetic Complications	Hypoglycemic Episodes
Poulain 2007 [135]	15 CD and T1D	before vs. after GFD	NS	before vs. after GFD: 0.9 ± 0.2 vs. 1.0 ± 0.4, p = 0.05	-	-
Rami 2005 [136]	98 CD and T1D vs. 195 with T1D only	CD and T1D vs. T1D only at diagnosis of T1D, diagnosis of CD and follow-up	NS	NS	-	NS
Sanchez-Albisua 2005 [137]	Five CD and T1D	before vs. after GFD	before vs. after GFD:8.0 vs. 7.3, p = 0.05	-	-	improved in two out of five compliant patients
Saukkonen 2002 [138]	18 CD and T1D	before vs. after GFD	NS	-	-	-
Sun 2009 [139]	49 CD and T1D vs. 49 with T1D only	CD and T1D vs. T1D only prior to and at diagnosis of CD as well as after 1 and 2 years on GFD	CD and T1D vs. T1D only: prior to and at diagnosis of CD: significantly lower in CD and T1D; NS after GFD	NS	-	-
Saadah 2004 [140]	21 CD and T1D vs. 42 with T1D only	before vs. after 1 year on GFD and comparison with patients with T1D only	CD and T1D vs. T1D only: NS; before vs. after GFD: NS	CD and T1D vs. T1D only: lower in CD and T1D prior to GFD (p = 0.054): NS after GFD	-	-
Valetta 2007 [141]	27 CD and T1D vs. 43 with T1D only	CD and T1D vs. T1D only at diagnosis of CD as well as after 1 and 2 years on GFD	NS	NS	-	-
Westman 1999 [142]	20 CD and T1D vs. 40 with T1D only	CD and T1D vs. T1D only; compliant vs. non-compliant to GFD	NS	-	-	-

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TG6 Auto-Antibodies in Dermatitis Herpetiformis

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Abstract: Dermatitis herpetiformis (DH) is an extraintestinal manifestation of gluten sensitivity, in which an autoimmune response is directed against transglutaminase 3 (TG3), an epidermal transglutaminase. TG2 is the autoantigen in celiac disease (CD), defined by the presence of enteropathy, and TG6 is the autoantigen in neurological manifestations of gluten sensitivity. The interplay between B cell responses to these 3 transglutaminases in developing the clinical spectrum of disease manifestations is not completely understood. Also, the individual or combined diagnostic and predictive value of the respective autoantibodies is not fully explored. We examined the prevalence of TG6 antibodies in a cohort of patients with DH. TG6 positivity was found in 13/33 (39%), with IgA detected in 11 patients, IgG in 3, and both in 1. This was significantly higher compared to what is seen in the classic CD cases (14%) in a Finnish population. TG6 positive baseline samples constituted 60% of DH patients with no enteropathy ($n = 10$), as opposed to 17% positivity in those with overt enteropathy ($n = 12$; Marsh IIIB). Repeat testing after adherence to a gluten-free diet for 1 year showed reduced titers for TG6 antibodies in 11/13 (85%), whereby 7 patients were now TG6 antibody-negative. Four patients seroconverted and tested positive for TG6 antibodies at one year, due to the ongoing exposure to gluten. We report another patient who presented with neurological manifestations (encephalopathy) leading to the diagnosis of CD, who was intermittently adhering to a gluten-free diet. Serological testing at baseline showed him to be positive for antibodies to all 3 transglutaminases. Eleven years later, he developed DH. He also subsequently developed ataxia and peripheral neuropathy. Although TG3 and TG6 autoantibodies are linked to certain disease manifestations, TG2, TG3, and TG6 autoantibodies can be present across the spectrum of GRD patients and might develop years before onset of symptoms of extraintestinal manifestations. This is consistent with gluten-dependent adaptive immunity being a necessary but not sufficient pretext to organ-specific damage. TG6 antibodies appear to develop more frequently in patients where tolerance to gluten was broken but, either there was no development of the molecular state driving the tissue destruction at the level of the gut, or perhaps more likely, there was more resistance to developing this phenotype.

Keywords: transglutaminase antibodies; TG2; TG3; TG6; dermatitis herpetiformis; gluten ataxia; celiac disease; gluten encephalopathy; gluten neuropathy

1. Introduction

Gluten-related disorders (GRD) are a group of immune-mediated diseases with diverse manifestations, triggered by the ingestion of gluten [1]. Enteropathy/celiac disease (CD) is not a prerequisite for the diagnosis of GRD, and some patients exclusively present with extraintestinal manifestations in the absence of enteropathy. Such manifestations include skin involvement in the form of dermatitis herpetiformis (DH) and a diverse range of neurological dysfunction, including cerebellar ataxia, sensorimotor axonal neuropathy, sensory ganglionopathy, and encephalopathy characterized by headaches and cognitive difficulties, often with white matter abnormalities on brain imaging [2].

The identification of TG2 as the autoantigen in CD was an important step in our understanding of the pathophysiology of CD [3]. Assessment of serum anti-TG2 antibodies has since become an important tool in CD diagnosis, as a surrogate marker of disease [4]. Recent success in recapitulating the hallmark features of CD including villous atrophy, plasmacytosis, and anti-TG2 autoantibodies in a mouse model shed light on the interplay between gluten, genetics, and IL-15 driven tissue inflammation in the establishment of CD pathology [5]. Importantly, these studies revealed how overexpression of IL-15 leads to activation of intraepithelial cytotoxic T cells, thereby providing a mechanistic explanation regarding the absence of intestinal tissue destruction ('normal' gut mucosa), even in the presence of adaptive gluten immunity in some patients. However, despite these advances, the mechanism underlying the clinical spectrum of GRD remains incompletely understood [1]. Variations in the specificity of antibodies produced in individual patients appear to be linked to specific extraintestinal manifestations. The epidermal transglutaminase 3 (TG3) was shown to be the autoantigen in DH [6]. The discovery of another transglutaminase primarily expressed in neural tissue (TG6), that shared enzymatic properties with both TG2 and TG3, offered further insights into the pathophysiology of neurological manifestations of GRD [7]. Patient-derived autoantibodies to these different isozymes are not crossreactive [7,8], and their development appears to be linked to the shared enzymatic properties of these enzymes rather than their structural similarity and potential shared epitopes (for review see [9]). This, therefore, suggests that any of these transglutaminases could be the primary immunological target of the gluten-driven immune response, although relative abundance in the gut and sensitivity to regulation by proinflammatory mediators explains the unique association of TG2 with GRD development.

The potential interplay between B cell responses to these 3 transglutaminases in disease manifestation and individual or combined diagnostic and predictive utility of respective autoantibodies was not fully explored. Here, we examine the prevalence of TG6 antibodies in a well-characterized cohort of patients with DH, and we discuss the implications of the findings for interpretation of patient serology in the wider context of GRD. We also discuss an interesting clinical case that highlights the potential predictive value for these antibodies.

2. Methods

2.1. Patient Selection

Serology samples from 33 DH patients diagnosed with granular IgA deposits on skin immunofluorescence biopsy were collected at diagnosis, and then 6 months and one year after a gluten free-diet (GFD) at the Tampere University Hospital; 31 patients adhered to the GFD. All patients provided informed consent and the project was approved by the local ethics committee (no specific code was allocated). The project adhered to the ethical principles for medical research, according to the Declaration of Helsinki. Small bowel biopsy was taken at diagnosis and was graded histologically as subtotal or partial villous atrophy with crypt hyperplasia and increased intraepithelial lymphocytes (24 patients), or normal mucosa (9 patients). Sera of a control group consisting of 27 patients with atopic dermatitis and 9 patients with psoriasis were also analyzed.

2.2. Serological Testing

Determination of anti-TG6 IgA and IgG was done using our in-house ELISA assays. The methodology was described in detail elsewhere [7,10]. Full-length human TG6 was produced in SF9 cells and diluted to 2 µg/mL in 20 mM Tris/HCl, 300 mM NaCl, pH 7.6, for coating of high-capacity protein binding 96-well plates (Immulon^{TM} 2 HB). All steps to reveal antibody binding were performed according to the published procedure. All serum samples were analyzed in duplicate, on wells containing antigen or only BSA, included on the same plate. The BSA-only background was subtracted from the values for antigen, and the units were calculated from a series of standards run in parallel, whereby a measurement >75 U/mL for IgA or >34 U/mL for IgG was considered to be positive. Standards were calibrated to be consistent with those used in commercial assay produced by Zedira (2nd generation assay). Results are given as the mean of two independent determinations.

3. Results

The clinical characteristics of the cohort of patients with DH were already published [11]. In brief, TG3 antibody positivity was seen in 88% (29/33) as compared to 24% (19/79) in patients diagnosed with classic CD. Endomysial antibody (EMA) positivity was found in 79% (26/33) of the DH group. The percentage TG3 antibody positivity dropped from 86% (24/28) to 21% (6/28), after a year on strict gluten-free diet.

In the current study, TG6 antibody positivity was found in 13/33 (39%) of DH patients (Figure 1). Eleven were positive for IgA anti-TG6, 3 for IgG, and 1 for both. Sera from patients with unrelated dermatological conditions (36) were also investigated as a control, with one psoriasis patient, testing positive for TG6 IgA (3%). Interestingly, tissue destruction, as determined by the histopathology of intestinal biopsies showed an apparent inverse correlation to serum findings, with TG6 autoantibodies detected in the baseline samples of 6/10 (60%) patients with DH without enteropathy, as opposed to 5/11 (45%) with partial villous atrophy (Marsh IIIA), and 2/12 (17%) in those who had overt enteropathy (Marsh IIIB). Three patients with TG6 antibodies (23%) were serologically negative for anti-TG2 IgA (ELISA & EMA), which is similar to 7/33 (21%) in the cohort as a whole. However, all TG6-positive DH patients also had circulating TG3 IgA.
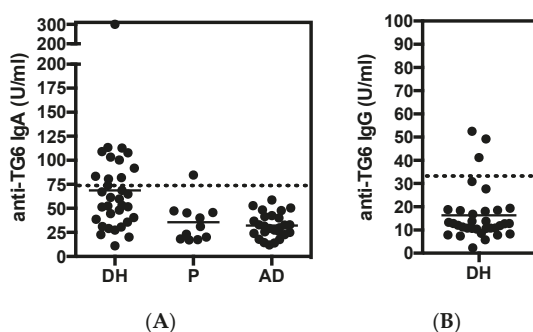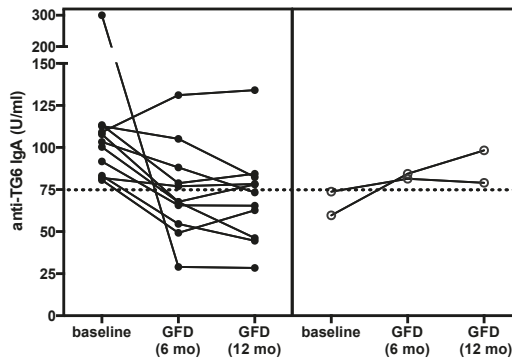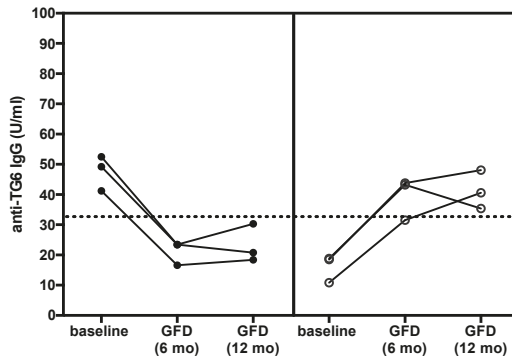

Figure 1. Serum concentration of (**A**) anti-TG6 IgA and (**B**) anti-TG6 IgG in the Dermatitis herpetiformis (DH) cohort (*n* = 33) at baseline. The cut-off limits are indicated by the dashed line. Samples from patients with psoriasis (P; *n* = 9) or atopic dermatitis (AD, *n* = 27) were included as non-Gluten-related disorders (GRD) controls. Note, for one AD sample, no result could be obtained due to unacceptably high non-specific reactivity in the assay.

Repeat testing after adherence to a gluten-free diet (GFD) for 6 months and 1 year (strict *n* = 28, partial *n* = 3) showed a response in 11/13 (85%) patients (Figure 2). Seven patients who were positive at baseline, tested negative for TG6 antibodies, after 1 year on GFD. One of the two patients that failed to respond to the GFD, also remained positive for anti-TG2 and anti-TG3 IgA, whereas the other patient

was only partially compliant with the diet, and although this patient became negative for deamidated gliadin peptide (DGP) antibodies he had persistently high titers of anti-TG3 IgA. Despite this evident effect of gluten withdrawal, and somewhat unexpectedly, the overall TG6 antibody positivity at one year was 8/31 (26%). This was because there were 4 patients who had seroconverted to become positive at one year, 2 for anti-TG6 IgA, and 2 for IgG (Figure 2). This appeared to correlate with ongoing exposure to gluten, as indicated by the other serology (DGP) in two patients (1 IgA and 1 IgG), while no obvious explanation could be found in the other two.

Figure 2. Longitudinal analysis of serum concentration of (**A**) anti-TG6 IgA and (**B**) anti-TG6 IgG in DH patients that tested positive for these antibodies at baseline on a gluten-free diet (GFD) (left panel, closed circles). Patients who tested negative at baseline but subsequently developed antibodies are given in the right panel (open circles). Note—two patients with TG6 IgA failed to respond to GFD, one of which also became positive for TG6 IgG after 6 months of GFD.

Given the high prevalence of anti-TG6 autoantibodies in this cohort of DH patients, patient records were retrospectively investigated for relevant history. Of the 31 DH patients that were analyzed longitudinally (GFD), six were dead and 25 were followed up to 2011 (mean 21 years), through questionnaire and hospital records. One death from Alzheimer disease was recorded but no other neurological conditions were found in any patient. Autoimmune diseases occurred in four, three had thyroid disease, and one had type 1 diabetes mellitus.

4. Predictive Value of Different Transglutaminase Antibodies: An Illustrative Case

A 41-years old man presented to neurology in 1997, with intractable headaches for the previous 2 years. He described them as severe, often unilateral and throbbing, lasting for several days and often associated with focal but transient neurological deficits (e.g., hemisensory disturbance and double vision). He also complained of memory difficulties and inability to concentrate. Brain MRI was done to rule out any ischemic cerebrovascular events. This showed white matter changes not typical of stroke or inflammation but of undetermined clinical significance (Figure 3). He had no vascular risk factors. He was started on aspirin and discharged. Subsequent outpatient clinical review showed ongoing symptoms of headache, and poor concentration and compromised memory function interfering with his everyday activities. Additional investigations at that stage ruled out systemic lupus erythematosus (SLE) and antiphospholipid syndrome. Cardiac echo and 24-h cardiac recordings, as well as vascular imaging were normal. Blood tests (available at that time) found him to be positive for anti-gliadin (AGA) and EMA antibodies. Duodenal biopsy confirmed the presence of gluten-sensitive enteropathy. It was thought that the headaches and MRI changes were secondary to CD (gluten encephalopathy) [12]. The patient was given advice for GFD by an experienced dietitian. He was followed up at regular intervals (every six months). Initial review after being on the diet for 6 months showed significant improvement in his headaches and cognitive difficulties. His adherence to the GFD over subsequent years was intermittent for a number of reasons—he could not afford gluten-free products, family problems and housing issues, and a one-year spell in prison. During this period, his antibody profile remained positive. He continued to be reviewed by the dietitian and attempts were made to ensure strict adherence to GFD. In 2006, he completely abandoned the GFD, but he restarted it a year later. He gave up the diet again in 2009. A few months later, he presented with an itchy vesicular rash over his arms and face. Dermatological review and skin biopsy confirmed the diagnosis of DH. He was still consuming gluten and serological testing for TG2 IgA, EMA, and anti-gliadin antibodies confirmed the presence of CD-related antibodies. He remained symptomatic with frequent headaches. More recently, he developed a degree of gait incoordination and a tendency to fall. He also complained of distal sensory disturbance with a burning feeling in his feet, less so in his hands. Further brain imaging showed evidence of cerebellar atrophy (Figure 4) that was not present in the baseline scan. Neurophysiological assessment including thermal threshold studies confirmed the presence of small fiber neuropathy.

Serum from this patient was stored at the time of the diagnosis of gluten encephalopathy (1998), and was subsequently available for testing for TG2, TG3, and TG6 autoantibodies, when these serological tests became available [7,13]. The tests showed him to be positive for deamidated gliadin peptide antibodies (IgA/IgG) and, interestingly, all 3 types of transglutaminase autoantibodies (TG2 IgA/IgG, TG6 IgA, and TG3 IgA/IgG). The TG2 antibody positivity would be expected on the basis of CD. TG6 antibody positivity would be in keeping with the diagnosis of gluten encephalopathy and the subsequent development of cerebellar ataxia (gluten ataxia) and neuropathy (gluten neuropathy), due to poor adherence to a GFD. The positive anti-TG3 antibody result from the 1998 sample would explain the subsequent development of DH, which however, manifested in 2009, over 10 years after the initial presentation with the neurological complaints. The patient is still under regular review and during his last attendance (August 2020) he tested positive for TG2, EMA, AGA, and TG6 IgG and IgA antibodies. AGA and TG2 antibodies were tested throughout his clinic appointments and were always positive. An observed reduction of the TG2 titer was only seen in 2008 and 2016, whilst he was trying to be strict with his GFD.
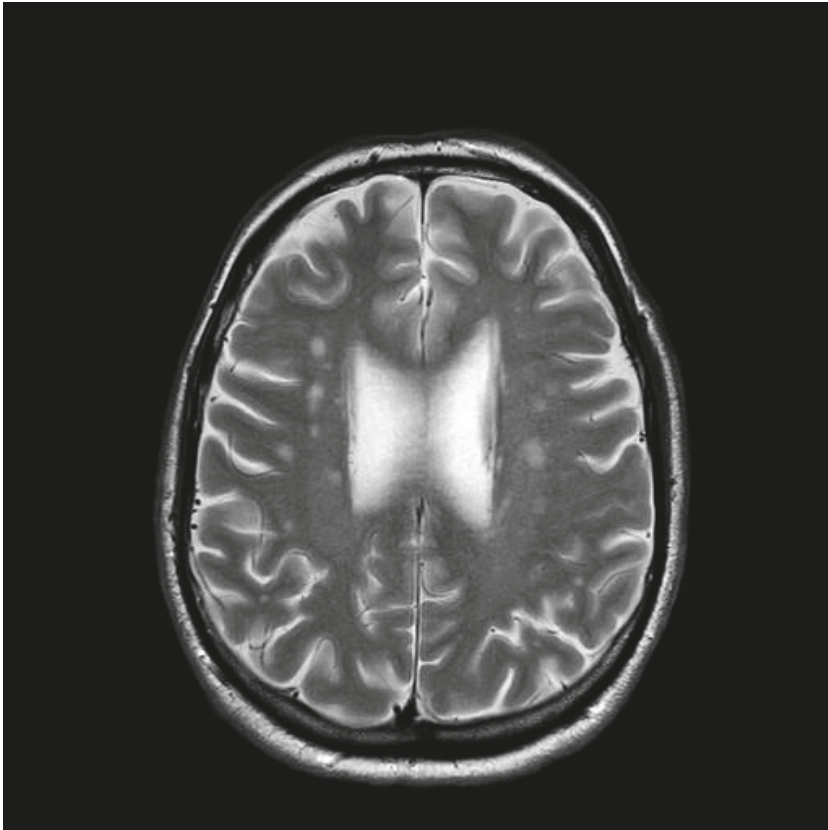
Figure 3. Brain Magnetic resonance imaging (MRI) scan conducted on presentation in 1997. The patient was diagnosed with gluten encephalopathy, having presented with headaches and cognitive difficulties. Serological testing and subsequent duodenal biopsy confirmed celiac disease (CD). The scan shows white matter changes typical of what is seen in the context of gluten encephalopathy.
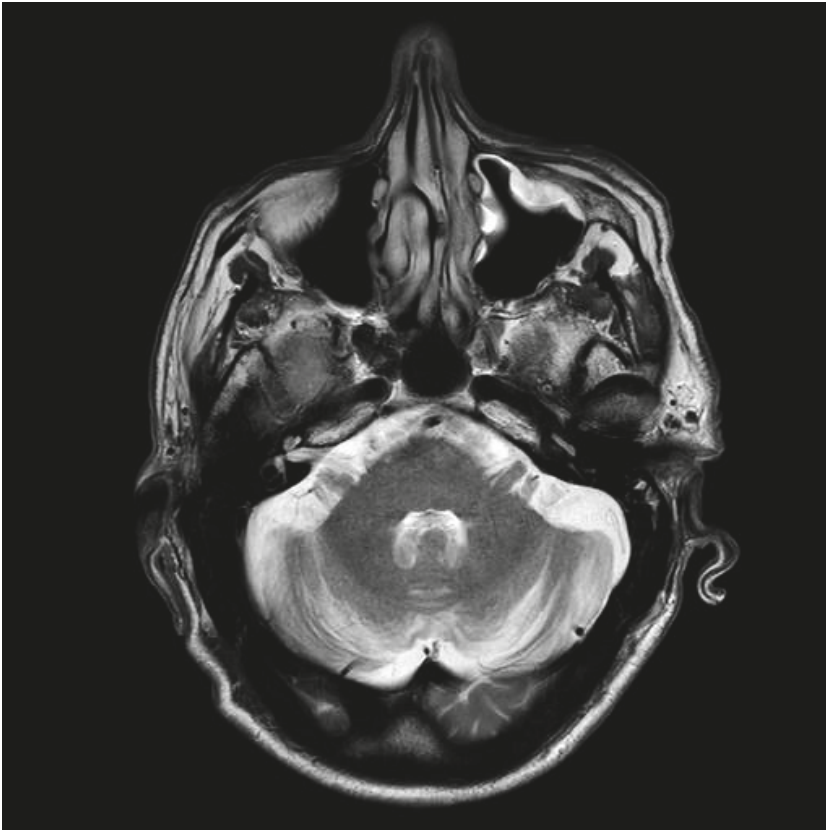
Figure 4. Brain MRI scan conducted on the same patient, as in Figure 3. The scan was conducted in 2018 and at that point showed evidence of cerebellar atrophy that apparently developed over an interval of 21 years. The patient now displays clinical evidence of cerebellar ataxia.

5. Discussion

The significance of the serological presence of TG2, TG3, and TG6 antibodies amongst different populations of patients with gluten-related disorders needs clarification. Here, we report a high prevalence of circulating anti-TG6 autoantibodies in DH patients (39%), which was an unexpected finding. It supports the notion that GRD patients, independent of clinical presentation may produce any of these TG isozyme-specific antibodies, or indeed any combination thereof. However, we previously showed that the prevalence of circulating TG6 antibodies in CD patients presenting with ataxia in the UK was much higher than in classic CD patients (73% vs. 40%) [14]. Using the same methodology, the prevalence of TG6 antibodies in Italian pediatric CD patients presenting to gastroenterologists was found to be 25% [15]. Furthermore, in this pediatric cohort of CD patients, a significant correlation between duration of gluten exposure, before the CD diagnosis, and anti-TG6 prevalence/concentration was found [15].

The TG6 antibody prevalence in these 3 groups (patients presenting with neurological manifestations, adult classic CD patients, and pediatric CD patients presenting to the gastroenterologists) is analogous to what was observed in patients with DH. Circulating TG3 antibodies (DH-specific epidermal autoantibodies) were found in up to 87% of patients with DH but in only 24% and 11% of adult and pediatric classic CD patients, respectively [16]. It is noteworthy that in untreated patients

with DH, not all patients have circulating anti-TG3 antibodies, yet 100% have IgA-TG3 deposits in the papillary dermis, the site of the primary manifestation [6]. Therefore, although the presence of these antibodies (to TG2, TG3, and TG6) in the serum is diagnostically helpful, their absence does not preclude a localized response at the level of the target tissue (gut, skin, and brain).

Significant advances in understanding of the events leading to autoantibody development were made over the last decade. DH patients were recently shown to have TG3-antibody secreting plasma cells at the level of the gut. Importantly, a gluten challenge of such patients revealed that gluten exposure drove rapid expansion of the TG3-specific plasma cell population in the lamina propria, and their frequency correlated with the serum titer of the corresponding autoantibodies [17]. Furthermore, there was no evidence that this cell population recognizes TG isozymes other than TG3 [17]. This is in keeping with the results from the analysis of patient-derived immunoglobulins, indicating that cross-reacting antibodies are rare [7,8]. Similarly, we analyzed GRD patients presenting with classical CD or gluten ataxia for TG6-specific plasma cells and demonstrated their presence in the lamina propria (Aeschlimann, Dos Reis, Hadjivassiliou, unpublished results). Hence, it is likely that antibodies are generated at the level of the gut; not just TG2 antibodies but also autoantibodies to other TG isozymes. All TG isozymes implicated in GRD form stable thioester complexes with gliadin peptides [18], leading to the uptake and ultimately the presentation of MHC-gliadin complexes by B cells expressing the respective TG-specific IgD. Their activation can consequently be driven through interaction with gluten-specific T cells. In vitro studies confirmed that this was possible [19], and this explanation is consistent with the gut resident T cell response and exquisite gluten dependence of TG autoantibody production, as demonstrated here for TG6 antibodies in DH patients. Enhanced intestinal permeability might drive the immunological reactions that lead to antibody development and circulatory presence [20,21].

How the development of adaptive immunity leads to extraintestinal manifestations remains a matter of debate, although some evidence that the autoantibodies themselves play a role in this has been put forth [9]. Perivascular antibody deposition, as observed in GRD patients can drive organ-specific inflammatory processes that result in tissue damage. TG3 antibodies within immune complexes formed in the papillary dermis of DH patients retain enzymatic activity and thereby drive the innate immune cell activity [22]. Importantly, the demonstration that circulation-derived anti-TG3 antibodies can induce a dermatitis herpetiformis-like pathology in human skin-grafted SCID mice, supports a central role for TG isozyme-specific antibodies in disease establishment in different organ systems [23]. However, expression of anti-TG2 antibodies by themselves did not precipitate CD-like lesions in the small intestine or overt systemic manifestation akin of GRD [24], identifying that establishment of overt tissue damage might require failure in more than one immune regulatory system. The adaptive immune response is a prerequisite for the development of intestinal villous atrophy but is not by itself sufficient. An interplay with the innate immune system that drives IL-15 overexpression in two distinct tissue compartments in the gut is required to mediate tissue destruction [5]. Perhaps this paradigm also applies to the extraintestinal manifestations, with development of anti-TG3 or anti-TG6 antibodies being required for the respective organ-specific manifestations but not being sufficient to precipitate overt tissue damage. This notion is consistent with the observation that the 3 types of autoantibodies could occur across the different forms of GRD, but without apparent clinical correlation. Despite high prevalence of anti-TG6 antibodies in the DH cohort, retrospective analysis of patient history did not reveal any related neurological problems, as was the case in the pediatric CD patients with the anti-TG6 antibodies analyzed previously [15]. We also present a case report that illustrated that the circulating antibodies could indeed be present for long periods of time (>10 years), prior to the onset of the corresponding extraintestinal manifestations. Late onset of DH was also reported in patients that initially presented with classical enteropathy, and appeared to be connected to intermittent GFD (antigen re-stimulation), as was the case here [25,26]. Extraintestinal manifestations were, however, not the consequence of reaching a threshold in the circulating antibody concentration, as there was no correlation between serum concentration and clinical presentation.

Of interest is the potential significance of these antibodies when present in patients who do not seem, clinically at least, to have any obvious corresponding extraintestinal dysfunction. Indeed, our recent work demonstrated that the subgroup of patients with classical CD who are anti-TG6 antibody positive (40% of UK patients) already show a significant reduction in the volume of specific brain regions, when compared to those who are TG6 antibody negative [27]. This finding also suggests that careful investigation (neurological examination and brain imaging) might reveal neurological deficits that are largely ignored, either because they are not considered by gastroenterologists or because the patients would not discuss neurological symptoms in the context of a gastroenterology consultation. Long-term studies of seemingly asymptomatic individuals screened for GRD (e.g., because of family history) who are serologically positive for these transglutaminase autoantibodies, might be helpful in understanding their lifelong significance and help inform the advice given to such patients.

Molecular etiology might also explain the observation that extraintestinal manifestations appear to be a late phenomenon. For example, patients with gluten ataxia and CD presenting with ataxia have a mean age at presentation of 53 years, as opposed to the mean age of 43 years, observed in patients presenting with the classic CD symptoms to gastroenterologists [28]. The prevalence of anti-TG6 antibodies in this Finnish DH cohort was 39%. This figure differed to what was found to be the prevalence of TG6 antibodies in a Finish cohort (same geographical area as the DH patients) of patients presenting with classical CD, which was 12/86 (14%) [14]. Furthermore, we made the observation that TG6 antibody positivity was more prevalent in those DH patients without enteropathy (60%), when compared to those DH patients with overt enteropathy (17%). This is in line with a significant proportion of patients with gluten ataxia and circulating TG6 antibodies displaying none or only minor signs of intestinal tissue damage [14]. TG6 antibodies, therefore, appear to be developed more frequently by patients who lost oral tolerance to gluten but either did not develop the molecular state that leads to tissue destruction at the level of the gut, or perhaps more likely, were more resistant to developing this state due to their genetics.

There were limitations to this study. Ideally, serological testing for all different GRD markers should take place without intermittent sample storage. Longitudinal analysis, as carried out here, is always a compromise between analysis of samples at the point of collection or at a later point when all linked samples for a cohort are available for simultaneous analysis; the latter approach was taken here. In our hands, there is no indication for sample storage (even long-term) affecting measurements of TG6 autoantibodies, as long as the samples were kept in sealed tubes for storage and repeated freeze–thawing cycles were avoided. Such sera are not easy to collect particularly from patients with DH who are becoming increasingly rare. The case described above is a single example of what we observed, and ideally, it should be followed up in a large cohort of patients who do not adhere to a GFD. However, such cases are not common and most patients with CD are likely to adhere to a GFD. Nevertheless, we believe that individual case reports can provide important insights.

6. Conclusions

We demonstrated here for the first time that TG6 antibodies are prevalent in patients with DH, and occur at a much higher frequency than what is seen in patients with classical CD. It appears therefore that although linked to certain disease manifestations, TG2, TG3 and TG6 autoantibodies can be present across the spectrum of GRD patients and may develop years before the onset of symptoms of extraintestinal manifestations. Nevertheless, our findings suggest that these antibodies could have a predictive diagnostic value for the future development of specific extraintestinal manifestations. Such autoantibody positivity at diagnosis might further inform the decision to adopt a GFD, particularly for those patients that appear to be asymptomatic but are at risk of developing neurological dysfunction in the longer term.

Author Contributions: M.H., T.R., and D.A. conceptualized the project. M.H. looked after the patient reported. T.R. and K.H. looked after all D.H. patients and collected the sera. P.A. and D.A. carried out the autoantibody

measurements. M.H. and D.A. produced the draft and all authors contributed and approved the final manuscript. All authors read and agreed to the published version of the manuscript.

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Review

Gluten and Autism Spectrum Disorder

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Abstract: An expanding body of literature is examining connections between Autism Spectrum Disorder (ASD) and dietary interventions. While a number of specialist diets have been suggested as beneficial in ASD, gluten has received particularly close attention as a potentially exacerbating factor. Reports exist suggesting a beneficial effect of the gluten-free diet (GFD) in ameliorating behavioural and intellectual problems associated with ASD, while epidemiological research has also shown a comorbidity between ASD and coeliac disease. However, both caregivers and clinicians have expressed an uncertainty of the value of people with ASD going gluten-free, and as the GFD otherwise receives considerable public attention a discussion which focuses specifically on the interaction between ASD and gluten is warranted. In this review we discuss the historical context of ASD and gluten-related studies, and expand this to include an overview of epidemiological links, hypotheses of shared pathological mechanisms, and ultimately the evidence around the use and adoption of the GFD in people with ASD.

Keywords: Autism Spectrum Disorder; gluten sensitivity; celiac disease; coeliac disease; review

1. Motivation and Literature Search Methods

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterised primarily by deficits in social communication and restricted/repetitive patterns of behaviour (DSM-5) [1]. As the name implies its phenotype exists on a spectrum, and overall it is estimated to affect as many as one in 69 children [2]. Interest in the use of specialist diets in ASD is increasing, as a way to alleviate its behavioural and intellectual outcomes. Though many dietary interventions have been suggested, the gluten-free diet (GFD) is among the most notable. Clinically, the GFD is well recognised as the primary treatment for patients with a gluten-related disorder. The most prominent of these is coeliac disease (CD) which is predominantly expressed as a gastrointestinal (GI) condition. However, physiological sensitivity to gluten is known to exist in other forms. These include other immune-mediated disorders (e.g., dermatitis herpetiformis and gluten ataxia), allergic reactions (wheat allergy), and non-coeliac gluten sensitivity (a condition characterized by self-reported gastrointestinal and extra-intestinal symptoms subjectively improving upon a GFD in subjects in whom other major organic gluten related disorders have been excluded) [3]. The clinical utility of the GFD outside of these contexts is debatable, and elsewhere its adoption within the general public as a sometimes "fad" diet heightens such scrutiny [4]. However, generally increased rates of GI problems have been reported in people with ASD, as has evidence of an apparent comorbidity between ASD and CD specifically. As adoption rates of specialty diets which include a gluten-free component are very high in ASD we were motivated to conduct a literature review which focused specifically on the interaction between ASD and gluten.

Searches were made on Pubmed on the 12th of November 2020. Terms included:

- Autism coeliac
- Autism celiac
- Autism gluten
- Autism wheat
- Autistic coeliac
- Autistic celiac
- Autistic gluten
- Autistic wheat

These terms were designed to capture a range of relevant terminology, for example "autism spectrum disorder" or "gluten free diet" would each be picked up by these. Regional variation in spelling of coeliac/celiac would also be accounted for. This returned 237 unique articles. The abstracts of all of these were read to determine eligibility for inclusion in the main review. Criteria for this were that the paper must be original research (i.e., not another review or systematic review) which in some way directly explored links between ASD and GRDs, the use of the GFD in ASD, or any other relevant interaction between ASD and gluten. Case reports were excluded, as were papers where the main article was not available in English. Seventy-nine articles were deemed eligible for inclusion. These were read, and any additional relevant citations found through this were added for discussion. Figure 1 shows these included papers according to their year of publication.
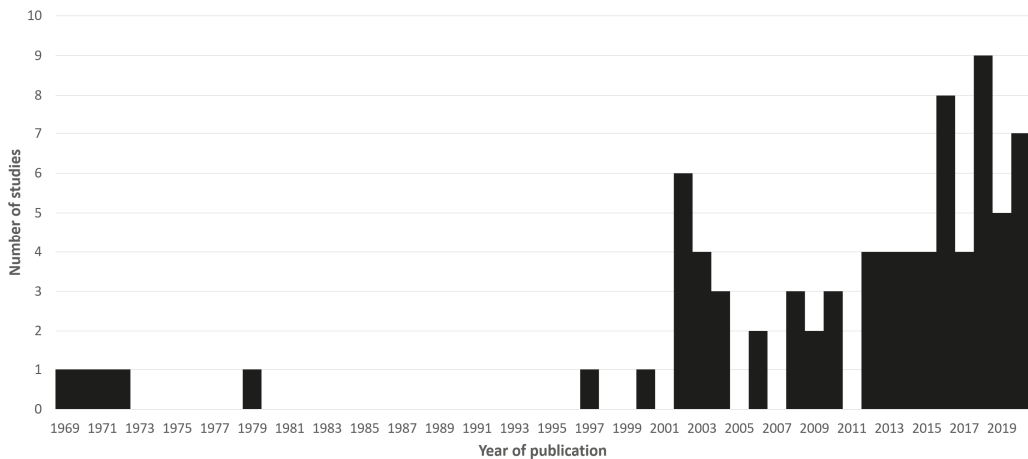

Figure 1. A histogram of eligible papers found in the primary pubmed search according to their year of publication.

Throughout the study of the literature, common research methods/sub-topics were noted. The remainder of this review synthesises these papers according to those themes. Additional literature was searched for and referenced where necessary to elucidate on a relevant key concept which was not adequately covered by the initial searches.

2. Historical Context

The first observation of a possible link between gluten and ASD was reported in 1969 by Goodwin & Goodwin [5], who noted in a cohort of 65 children with ASD that one 6 year old boy also had CD. This child's subsequent treatment with a GFD appeared to improve outcomes relating to his ASD. It is relevant to note that at that time the prevalence of CD was considered to be far less than the 1-in-100 that it is sometimes reported as today [6], largely due to the lack of effective diagnostic methods such as serological testing. In a commentary piece on the then-emerging topic, Dohan references in their 1970 paper [7] that CD is

thought to affect approximately 1 in 3000 people, making suspicion after finding it in one of a cohort of 65 children with ASD understandable. Another point of interest emerges from this paper, which was purely focused on links between CD and schizophrenia but in which Dohan also found it relevant to include an anecdotal report of increased CD rates in ASD groups ("at least 2 coeliac patients among 140 severely autistic children"). While limited phenotypic similarities between schizophrenia and ASD are still discussed now, initial characterisation of the conditions involved such overlap that full clinical separation did not occur until 1980 with the publication of the DSM-III [8]. To a modern reader, this explains why there are a number of early articles which combine ASD with schizophrenia and otherwise draw potentially confusing links between the two.

Goodwin et al. published another study in 1971 [9] which is arguably the first trial investigating how gluten modified behaviour in a cohort of children with ASD. Also included were controls and a group of participants with schizophrenia for further comparison. Here, the participants followed the "sprue diet" (GFD) for a single day, in which they were also given a cherry drink which had added to it either gliadin or a placebo (sugar). They were then subsequently monitored and tested via investigation of blood counts and electrophysiological recordings by trans cephalic direct current. Although the authors note some findings, these predominantly focus on differences which appear to separate ASD and schizophrenia participants, providing only one comment on the effect of gliadin where it appeared to reduce plasma cortisol levels. However, this was observed across both control and ASD participants, and when coupled with the small sample sizes (the ASD group had 9 participants for that portion of the results) and extremely short period of dieting, it is difficult to extrapolate any meaningful conclusions. Further studies in the 1970's included a comparison of serum alpha-1-antitrypsin levels between children with ASD vs. children with CD [10] (finding them comparably abnormal and suggesting a shared pathology), an experiment published in a book [11] describing 72 patients with ASD in whom CD was diagnosed (without biopsy) in 8, and another trial of gluten in eight children with ASD who already followed a GFD and were purportedly better for it [12]. This latter study saw the participants stop their diet to undergo a gluten challenge for 1 month, hypothesising this would worsen their phenotype, but finding no change in bodily measurements (weight, bowel habit etc.) or behaviour (measured by parental reports and observation from a specialist paediatrician).

To summarise, early interest in the topic was driven by essentially anecdotal reports of apparently comorbid cases of CD with ASD. Direct experimentation of this resulted in largely negative findings. These studies had small samples sizes and, when viewed with a contemporary lens, suffered from experimental designs and measurement techniques which would now be considered extremely insensitive in targeting relevant outcomes. Following these papers, little relevant research activity appeared until the mid 1990's when the topic appeared to become more popular once again.

3. Gastrointestinal Symptoms in ASD

A heightened rate of gastrointestinal (GI) symptoms in people with ASD is well documented. While this particular topic in its entirety falls outside of the scope of the present systematic review, the observation of these symptoms is a major motivator for gluten-specific research. Relevant studies from the review are therefore included here, as well as other key literature.

In 2014 Chaidez et al. [13] conducted a large study in which 499 children with ASD were compared to typically-developing (TD) children ($N = 324$) and children with developmental delay ($N = 137$) in terms of GI symptoms measured by 10 Likert scales (abdominal pain, constipation etc.). After controlling for age, sex, maternal education and medications which may lead to GI side effects, children with ASD had significantly heightened odds ratios (OR) compared to controls for 8 outcomes, the lowest being 3.14 (abdominal pain) and the highest being 8.61 (sensitivity to foods). The children with ASD and developmental delay were not significantly different from one another.

This is one of a number of similar studies who's findings are supported by meta-analyses; a pubmed search of "gastrointestinal autism" found the most recent meta-analysis was performed in 2014 [14]. This included 15 studies which gave a combined sample of 2215 children with ASD in which four variables were included; general GI concerns, diarrhoea, constipation and abdominal pain. Each of these was found to be significantly more prevalent in the ASD group compared to TD children, with overall OR's of 4.42, 3.63, 3.86 and 2.45 respectively.

Relevant studies from the current review include a report [15] of a higher frequency of constipation in children with ASD, a study [16] which found children with ASD and regression more often had abnormal stool than those without regression, and another experiment [17] which found GI symptoms to be more common in children and adolescents with ASD than in TD controls, and for these symptoms to be weakly correlated to behavioural measures. Correlations such as these have been documented elsewhere [18–20]. These studies reference the often non-specific nature of GI symptoms with one explaining that "a GI pathology specific to ASD had not been established" (Babinska et al., 2020 [17]). Regardless, a notable body of literature has investigated for a comorbidity between ASD and specific GI conditions, often finding significant results.

4. The Co-Morbidity between ASD and CD

Following early literature from the 1970's, the first study to investigate for an increased rate of CD in ASD was Pavone et al. in 1997 [21]. Pavone examined a cohort of children with ASD (*N* = 11) to detect the rate of CD (by antibody and ultimately biopsy testing), and similarly a cohort of children with CD (*N* = 120) to detect the rate of those with features of ASD (as reported by parents and according to the DSM III-R). None of the children with ASD had biopsy-proven CD, while none of the children with CD met criteria for a full ASD diagnosis (though a limited few did show isolated features). This was therefore overall a negative study, but the limitations of examining in such small cohorts as 11 are evident.

Since then, a limited number of large epidemiological studies have been conducted which generally do show an effect indicating CD and ASD to be comorbid to one another. One of 2009 [22] which focused specifically on comorbidities to ASD within parental medical history, used the Danish Civil Registration System to identify all children born between 1993 and 2004 with ASD (*N* = 3325). Here, maternal history of CD led to a significant, overall incidence rate ratio (IRR) of 2.97 in terms of the child having ASD. Other studies examining comorbidities within the same participant have also found significant results while using medical databases. A 2017 study [23] examined for the risk of psychiatric sequalae in children with CD (*N* = 10,903), finding a hazard ratio (HR) of 1.5 (univariate analysis) of being diagnosed with ASD after their CD diagnosis but before the age of 18 (adult data was not included). The same research group has replicated this more recently [24] with a larger cohort of people diagnosed with CD while a child (*N* = 19,189), but which this time did also include psychiatric diagnoses obtained after 18. Here, the HR of developing ASD was 1.47, the highest of all disorders included in analyses.

These papers do however contrast an earlier study, again by the same research group [25], which examined specifically for the likelihood of an ASD diagnosis *preceding* a CD diagnosis in children and adults with CD (*N* = 26,995). This OR was non-significant, though potentially of interest was a finding wherein previous ASD was still associated with an increased risk of having normal mucosa on biopsy, but positive CD serological test results (tissue transglutaminase; TTG, endomysial; EMA or gliadin; AGA antibodies, reported as a single grouping). While the immediate clinical implication of positivity to these antibodies varies, i.e., TTG/EMA positivity indicates CD with high sensitivity/specificity while many generally-healthy individuals may exhibit AGA positivity, it should be highlighted that within the study of wider "gluten sensitivity" heightened rates of any of these may be considered pathologically-relevant when compared to an appropriate "control" such as in this discussed study. This is therefore an important study as it highlights the link between serological markers of gluten sensitivity and ASD in the absence of enteropathy.

Other prevalence research has been conducted with less stringent diagnostic criteria and/or smaller sample sizes, finding mixed results. Studies with significant findings include Calderoni et al. [26] who examined a cohort of children with ASD (*N* = 382) and found the rate of CD within the sample was 2.62%, although it should be noted this sometimes relied only on a positive serological (TTG/EMA) result and formal CD diagnosis was not always confirmed. Valicenti-McDermott et al. [16] found an increased family history of CD and/or inflammatory bowel disease in children with ASD who also exhibited regression (*N* = 24), compared to children with ASD without regression (*N* = 71). In a letter to the editor, Barcia et al. [27] report an experiment where of 91 "randomly selected" children with ASD, 4 had "biopsy-proven" CD (the authors reference diagnostic guidelines for diagnosis where Marsh grade 3 denotes CD). This is a rate of 4.4% which is considerably higher than might be expected. Mazzone et al. [28] found in a cohort of 100 children with CD that 2 had ASD (while none of a control group did); whether this is a positive result or not is arguable.

Studies with negative findings include Alabaf et al. [29] who via parental reporting of 91 children with ASD did not find an association with CD (not reported but this was measured, implying a negative finding). Juneja et al. [30] screened children with ASD (*N* = 150) for CD defined by IgA TTG testing, finding no positive tests. In 2012, Batista et al. [31] examined children and adolescents with either ASD (*N* = 147) or biopsy-proven CD (Marsh grade 3, *N* = 211) for the rate of the other. The ASD group was found to be entirely negative for CD (although one subject did have a weakly-positive TTG result with negative EMA), while two cases of ASD were found in the CD group; this was concluded to not be above chance. Zelnik et al. [32] examined a cohort of CD patients (*N* = 111) for a range of neurological outcomes including ASD, although as this was reported mixed in with other learning disabilities and ADHD (which overall affected 20.7% of the group) how common ASD specifically was is not known. Finally, Black et al. [33] examined medical records to identify 96 children with ASD and reported on all diagnosed gastrointestinal comorbidities, failing to identify any CD cases.

A recent meta-analysis [34] combined some of the above studies (where eligible) to find a significant odds ratio of 1.53 in terms of CD patients having ASD, but a non-significant likelihood of ASD participants having CD. Overall therefore, the majority of studies relevant to the question of comorbidity have used variable sample sizes and diagnostic methods, making definitive conclusions difficult in most individual instances. However, the strongest powered are undoubtedly those from Sweden which studied large cohorts and established an increased risk of a subsequent ASD diagnosis in people with CD, therefore showing a convincing comorbidity. This is further supported by the meta-analysis finding, which showed the same. Studies which examine the "reverse" of this, where initial ASD features may increase risk of subsequent CD, have led to more negative findings however do demonstrate an association with the development of gluten antibodies in the absence of clinical CD. It should also be highlighted that a very large epidemiological study which principally studies an ASD cohort for the rate of CD is absent, which may introduce a sampling bias when interpreting the findings of this overall field. Overall therefore, ASD does appear comorbid to CD, and while an increased risk of CD in ASD is not currently supported a suspicion of ASD being linked to subsequent, immune-mediated "gluten sensitivity" may be warranted.

5. Hypothetical Mechanisms of Action

With a comorbidity between ASD and CD established, a natural question is of what shared pathophysiology may drive these associations. Further, as non-specific GI symptoms are also seen to be generally more prevalent in ASD and that gluten sensitivity is increasingly understood to be a spectrum that extends beyond the clinical criteria for CD specifically, it is important to consider any mechanism of action between gluten and ASD.

From an early point in the literature, hypotheses have frequently related in some way to heightened autoimmunity in ASD. While a predisposition towards autoimmunity was

noted as early as 1971 [35], enquiries of this nature gathered pace after a key publication in 2001 [36] which showed children with ASD (with regression) to have increased markers of innate and adaptive immune response (TNF-A, cytokines etc.). This was investigated at the time partially in response to parental reports of children with ASD suffering apparently high rates of reactions to dietary irritants, and this autoimmune phenotype was subsequently hypothesised to be part of the aetiology of ASD. The authors presented this idea in terms of environmental stimuli triggering an immune response which exacerbates ASD features, and in the specific case of their study hypothesised it may stimulate regression.

In the early 2000's a series of publications by Vojdani et al. [37–39] built on this evidence by focusing on more specific dietary triggers and hypothetical knock-on effects they would lead to in terms of molecular pathways. Here, focusing mainly on gliadin (a gluten-specific protein) and casein (a protein in dairy products), it was demonstrated that children with ASD have high rates of antibodies against these (i.e., anti-gliadin and anti-casein) as well as antibodies against DPP4, a digestive enzyme. DPP-4 is important in the processing of gliadin. Initially, gliadin is degraded into various peptides which include gliadinomorphin-7 [40], an immune reactive substance with "opioid activity", i.e., which stimulates opioid receptors in the body [41]. Further degradation of gliadinomorphin-7 is therefore required, which is where DPP4 functions by cleaving such peptides [42]. As Vojdani et al. reported, the existence of anti-DPP4 would hypothetically reduce the amount of circulating DPP4, increasing the abundance of gliadinomorphin-7 and the likelihood of downstream, opioid-like effects. It should be highlighted that casein and other dietary peptides are similarly degraded to intermediary substances with opioid properties (e.g., casomorphin [43]), and together these potentially harmful peptides have been termed "exorphins" [44].

Stimulation of the opioid system has been studied in the context of ASD features. An early proponent of this link, Panksepp outlined a theory in 1979 [45] (based largely on his earlier animal model experiments) wherein excess opioid activity may lead to the decreased social behaviour seen in ASD. This theory has persisted until today, with numerous publications concerned with evidence of opioid overactivity in people with ASD. Animal studies have continued to show the importance of a balanced opioid system in maintaining social behaviours which are similar to those impacted in ASD, while experiments investigating for levels of relevant, opioid-like peptides in the sera, CSF or urine of people with ASD have generally shown raised titres albeit with some notable exceptions where decreases have been reported [46]. Indeed, measurement of urine peptides has become a common tool in this field, with high levels being seen as an indication of insufficient digestion of food which may lead to excess exorphins [47].

An alternative theory has focused on the role that oxidative stress may play in ASD, which may lead to a state of inflammation in the brain. It has for example been reported that people with ASD have an impaired antioxidant defence in the cerebellum [48], while problems metabolising nitrous oxide (which may lead to increased oxidative stress [49]) has also been proposed as driver of ASD pathophysiology [50]. This holds a relevance to gluten sensitivity, where increased oxidative stress has also been demonstrated, for example as triggered by gliadin [51] or as demonstrated generally by raised markers of oxidative stress across untreated children with CD [52].

Studies have also noted the potential for shared genetic predisposition. One recent paper by Bennabi et al. [53] compared genotyping data between ASD and control cohorts, finding that the haplotype HLA-DRB1*11-DQB1*07 was more common in the ASD group, with this being more prevalent still in those ASD patients with the most pronounced behavioural symptoms. A different haplotype (HLA-DRB1*17-DQB1*02) was conversely more common in the control group. The *07 haplotype was therefore concluded to be potentially causative and the *02 one protective. Of relevance is that the *07 haplotype is additionally recognised as associated with CD, leading to a suggestion that there may be a sub-group of people with ASD holding a genetic risk for both [54]. However, other genetic research has produced negative findings, such as a meta-analysis of genome-wide

association studies [55] which did identify regions associated with ASD but noted only overlap between these and schizophrenia.

The potential of reactivity of antibodies to gluten products should be discussed. Antibodies against tissue-transglutaminase (TTG) have very high sensitivity and specificity in diagnosing CD, meaning that as a comorbidity has been demonstrated they may have a relevance in ASD pathology. These antibodies have been reported to lead to apoptosis of neuroblast cells in vitro [56]. Other antibodies which may indicate gluten sensitivity but not CD specifically include transglutaminase 6 (TG6) antibodies, which have been indicated in the diagnosis of gluten ataxia [57] (where the cerebellum is the primary site of damage), with this supported by animal research showing TG6 to be distributed throughout the central nervous system including brain regions such as the cerebellum and thalamus [58]. TG6 antibodies have been reported at a rate of 4.4% in a group of 77 children with ASD [59]; this experiment lacked a control group and as this is a relatively novel marker it is difficult to evaluate if this is abnormal. Finally, gliadin antibodies have been shown to react with brain blood vessel structures [60], show cross-reactivity with neuronal synapsin 1 [61], and to be associated with rates of depression in people with CD and healthy controls [62]. Gliadin antibodies have been measured across a number of studies in ASD, frequently finding them to be raised. Those found in the current review are summarised in Table 1.

Table 1. A summary of studies which have investigated if gliadin antibodies (AGA) are affected by ASD. *: full text not available.

Citation	Cohort (Describes the ASD Group Unless Otherwise Specified)	Finding (% Where It Was above an Abnormal Cutoff Given Where Possible)
Cade et al. (2000) [63]	150 children and adolescents	IgG AGA raised (87% of group)
Vojdani et al. (2003; the two publications by Vojdani et al. in 2004 also report gliadin antibodies, but use the same dataset) [37–39]	50 patients	IgG (44%)/IgA (46%)/IgM (36%) AGA raised compared to controls
Kawashti et al. (2006) [64] *	30 children	AGA raised (50% of group) compared to controls
Batista et al. (2012) [31]	147 patients	IgG/IgA AGA not different to controls
Lau et al. (2013) [65]	37 children	IgG AGA raised (24.2% of group) compared to controls, and particularly in those with a GI medical history
de Magistris et al., 2013 [66]	162 children	IgG AGA raised (25.3% of group) compared to controls, higher in both those on GFD and regular diets.
Józefczuk et al., 2018 [59]	77 patients	IgG AGA raised (27.3% of group)
Abdel-Maksoud et al. (2020) [67]	66 children	IgA AGA titre lowered compared to controls

The potential for antibodies and other irritants to travel from the gut to the brain is raised by a number of studies which have demonstrated generally inflamed/abnormal intestinal findings in ASD [68–71], and others showing compromised intestinal permeability specifically [66,72]. Indeed, one other publication [73] examined gene and protein expression of brain and intestinal tissue of human ASD subjects, finding evidence of impaired intestinal permeability in combination with altered blood-brain barrier integrity. It should be noted that not all studies support an impacted intestinal permeability [59,74], but nonetheless these phenomena raise the possibility of a gut-brain axis interaction being relevant in ASD. Here, a negative feedback loop between the brain and the gut would lead to exacerbation of both neurological and GI outcomes. Arguably most studies which have investigated how gluten can impact people with ASD may fall within this broader

concept, where irritants enter the bloodstream from the gut to cause downstream negative consequences for the brain. A loop may be completed if the effect on the brain leads to alterations of behaviour and appetite which may maintain or exacerbate the cycle [75].

In summary, pathological interactions between ASD and gluten have focused on opioid activity from improperly digested gluten products, inflammation caused by oxidative stress and/or reactivity with anti-gluten antibodies, and some indications of shared genetic factors. These hypotheses provide some explanation for the previously discussed comorbidity between ASD and CD and also make it appear reasonable that gluten may exacerbate bodily stress in other groups of people with ASD who do not have CD. However, it remains unclear to what extent ASD populations and sub-populations are affected, and to what degree these findings represent a unique interaction with gluten specifically or are a consequence of a generally-raised autoimmune profile in ASD.

6. Trials of the GFD in ASD

Establishing that gluten is potentially harmful for people with ASD leads to the question of if a GFD would then bring any benefits. After the early studies of the 1970's, the first trial which involved gluten in any capacity was conducted in 1990 and is detailed in two publications [76,77] by Knivsberg et al. Here, fifteen children with ASD in combination with abnormal urine peptide results engaged with a gluten and casein-free diet (GCFD) for four years. Measurements were generally taken at baseline, one year and four year time points, and included scales which characterised psychotic behaviour in children, psycholinguistic ability and fluid intelligence. However, this data was not all collected consistently (e.g., the psychotic behaviour measurements were not made at 4 years), and authors note variable dietary success between the children. Regardless, significant findings suggested improvement across multiple outcomes, including normalisation of urine peptides. The 1990's saw one other trial [78], the primary analyses of which concerned a group of 22 children with mixed spectrum disorders (the most common being ASD) who undertook a GFD for 5 months. Other groups were also examined, e.g., children with ASD already on a GFD took a gluten challenge, although these sample sizes were very small. Similar to the Knivsberg study, improvement in behavioural outcomes was noted in response to the GFD although no change in urinary peptide levels were seen.

The first randomised trial was conducted in 2002 [79]. Here, 20 children with ASD and abnormal urinary peptides were randomised into parallel groups to receive either the GCFD or a regular diet for 12 months. Following this, improvements were noted across behavioural and intellectual outcomes. A number of randomised trials have been conducted since and those that utilise an intervention that in any way involves gluten are summarised in Table 2.

Table 2. A summary of randomised trials which have in some way included a gluten-free diet as an intervention in treating ASD.

Citation	N Randomized & Comment on Groupings	Participants Blinded?	Diet(s) Tested	Duration	Any Main Outcomes Significantly Affected by Intervention?
Gonzalez-Domenech et al., 2020 [80]	*N* = 37; crossover design. Mixed children and adolescents with ASD, without allergies to gluten or casein. Everyone on gluten & casein-containing diet at baseline.	No	GCFD vs. regular diet	12 months (6 months per crossover block)	None; those tested included behavioural/cognitive measures (ERC = III, ATEC & ABC), and urinary beta-casomorphin as a marker of poor digestion of casein.

Table 2. *Cont.*

Citation	N Randomized & Comment on Groupings	Participants Blinded?	Diet(s) Tested	Duration	Any Main Outcomes Significantly Affected by Intervention?
Piwowarczyk et al., 2020 [81]	N = 66; parallel group. Children with ASD, without celiac disease/wheat allergy. 8 week, GFD run-in period before start.	No	GFD vs. regular diet	6 months	None; those tested included behavioural/cognitive measures (ADOS-2, SCQ, ASRS, VABS-2, LIPS), and Rome-III for GI symptoms.
Grimaldi et al., 2018 [82]	N = 30; parallel groups. Children with ASD who did not take dietary supplements. Baseline food diaries identified groups who already either followed GCFD or regular diets; randomization to receive the prebiotic mixture happened within these groups.	Yes	GCFD + "B-GOS" prebiotic mixture (vs. GCFD without B-GOS, vs. regular diet with/without B-GOS).	6 weeks	Improvement in behavioural scores (ATEC & AQ) in children on GCFD + the prebiotic mixture (not observed in those on GCFD alone). No significant results reported for EQ-SQ or SCAS-P. Physiological changes (urine spectra, faecal samples, were observed in response to the prebiotic mixture, both across dietary groups and between them.
Adams et al., 2018 [83]	N = 67; parallel groups. Children and adults with ASD. 2 month run-in of no special diet or supplements.	No	Various interventions, added accumulatively (vs. no diet/modifications). At the end of the trial, interventions included GCFD (for 155 days) + supplementation of vitamins, minerals, essential fatty acids, carnitine, digestive enzymes & taking of Epsom salt baths.	12 months	Improvement in behavioural/intellectual scores (RIAS non-verbal IQ, CARS, SAS Pro, VABS-II, PDDBI Composite, ATEC, ABC, SRS & SSP) Improvement in GI symptoms (measured by 6-GSI). Some changes to complete blood count and blood chemistry panel markers, fatty acid profile, vitamin levels, RBC elements, homocysteine, l-carnitine No changes in handgrip strength or C-reactive protein
El-Rashidy et al., 2017 [84]	N = 45; 3 parallel groups (2 dietary interventions, and a controls). Children with ASD	No	GCFD vs. ketogenic vs. regular diet	6 months	Improvement in behavioural/intellectual scores (CARS, ATEC); in both GCFD and ketogenic groups. Degree of change was not sig. different between these groups, but each appear markedly larger than change observed in the control group (this specific comparison does not appear to have been statistically evaluated)
Ghalichi et al., 2016 [85]	N = 80; parallel groups. Children and adolescents with ASD, not following any special diets.	No	GFD vs. regular diet	6 weeks	Improvement in behavioural scores (GARS-2). Improvement in GI symptoms (ROME III)

<div align="center">

Table 2. *Cont.*

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Citation	*N* Randomized & Comment on Groupings	Participants Blinded?	Diet(s) Tested	Duration	Any Main Outcomes Significantly Affected by Intervention?
Hyman et al., 2016 [86]	*N* = 14; crossover design. Children with ASD, without celiac disease or wheat/milk allergy. 6 week run-in period of GCFD.	Yes	GFD vs. CFD vs. GCFD vs. regular diet.	12 weeks (Alternating diets delivered in "blocks" where every participant did each diet one week at a time. This was repeated 3 times, totalling 12 weeks)	None; those tested included behavioural scales (CARSA, RRLRS) and physiological scales (Bristol Stool Scale).
Pusponegoro et al., 2015 [87]	*N* = 74; parallel groups. Children with ASD, with high levels of urinary I-FABP excretion (indicating heightened intestinal permeability)	Yes	GCFD vs. regular diet	1 week	No change in behavioural outcomes (AWPC) Worsening of gastrointestinal symptoms; significant in within-group analysis, but change in this measure was not different between groups. No change in urinary I-FABP
Navarro et al., 2015 [88]	*N* = 12; parallel groups. Children with ASD, without celiac disease or food allergies. 2 week GCFD run-in period.	Yes	GCFD vs. regular diet	4 weeks	None; formal statistics generally avoided due to small sample size, but trends were generally absent in all outcomes which included behavioural/intellectual measures (CPRS-R, ABC) and physiological measures (lactulose/mannitol recovery ratio for intestinal permeability, or GI symptoms on a non-validated questionnaire)
Johnson et al., 2011 [89]	*N* = 22; parallel groups. Children with ASD.	No	GCFD vs regular diet	3 months	None; those tested included behavioural scales (CBC, MSEL, blinded observations) and physiological measurements (likert scales RE constipation etc.). Isolated sub-scores of MSEL & CBC were sig., though authors note no consistent pattern and reject them
Whiteley et al., 2010 [90]	*N* = 73; parallel groups. Children with ASD	No	GCFD vs. regular diet	24 months; interim analyses at 8 and 12 months would reassign regular diet participants to receive GCFD for the remainder, if sufficient improvement was observed in GCFD group.	Improvement in behavioural/intellectual outcomes (ADOS, GARS, VABS), no change in ADHD-IV at the 8 month analysis; the control group was added to the diet at 12 months making 24 month data un-comparable.

Table 2. *Cont.*

Citation	N Randomized & Comment on Groupings	Participants Blinded?	Diet(s) Tested	Duration	Any Main Outcomes Significantly Affected by Intervention?
Elder et al., 2006 [91]	*N* = 13; crossover design. Children and adolescents with ASD, without celiac disease.	Yes	GCFD vs. regular diet	12 weeks (6 weeks per crossover block)	None; those tested included behavioural/intellectual measures (CARS, ECO and observation of in-home behaviour). Authors do note parental reports indicating potential improvements in individual children when on the diet. Parents otherwise performed poorly at guessing which period of time they had been given the GCFD foodstuffs.
Knivsberg et al., 2002 [79]	*N* = 20; parallel groups Children with ASD in additional to abnormal urinary peptides.	No	GCFD vs regular diet	12 months	Improvement in behavioural and intellectual outcomes (DIPAB, LIPS, ITPA, Reynells spraktest, MABC)

Findings described in the final column relate to any analysis which indicates with statistical significance that the intervention affected an outcome. 6-GSI; 6-Item Gastrointestinal Symptom Index, ABC; Abberant Behaviour Checklist, ADHD-IV; Attention-Deficit Hyperactivity Disorder—IV rating scale, ADOS; Autism Diagnostic Observation Schedule, AQ; Autism Spectrum Quotient, ASD; Autistic Spectrum Disorder, ASRS; Autism Spectrum Rating Scale, ATEC; Autism Treatment Evaluation Checklist, AWPC; Approach Withdrawal Problems Composite (a subset of the PDD-BI), CARS; Childhood Autism Rating Scale, CARSA; Conners Abbreviated Rating Scale and Actigraphy, CBC; Child Behavior Checklist, CPRS-R; Connor's Parent Rating Scale-Revised, DIPAB; Diagnose af Psykotisk Atfærd hos Børn, ECO; Ecological Communication Orientation, ERC-III; The Behavioral Summarized Evaluation, EQ-SQ; Empathy and Systemising Quotient, GARS; Gilliam Autism Rating Scale, GI; Gastro-intestinal, ITPA; Illinois Test of Psycholinguistic Abilities, LIPS; Leiter International Performance Scale, MABC; Movement Assessment Battery for Children, MSEL; Mullen Scales of Early Learning AGS edition, PDD-BI; Pervasive Developmental Disorders Behaviour Inventory, RIAS; Reynolds Intellectual Assessment Scales, RRLRS; Ritvo-Freeman Real Life Rating Scales, SAS Pro; Severity of Autism scale-Professional Evaulation, SCAS-P; Spence's Children Anxiety Scale-Parent version, SCQ; Social Communication Questionnaire, SRS; Social Responsiveness Scale, SSP; Short Sensory Profile, VABS-2; Vineland Adaptive Behavior Scale, Second Edition.

Examining this literature reveals a very mixed picture of findings. Of the 13 RCT's found in the current review, improvements of some kind were noted in 6 [79,82–85,90], no findings were observed in another 6 [80,81,86,88,89,91], while a worsening of GI symptoms (in response to the GCFD) was observed in one study [87]. All 6 studies which noted a positive effect from the interventional diet included improvements in intellectual/behavioural outcomes, and sometimes also in physiological measurements (e.g., GI symptoms).

Consolidation of these studies is difficult even beyond the mixed findings. One immediate observation is that the exact dietary intervention employed is variable. This increases heterogeneity between studies and makes commenting on the effect of gluten specifically impossible in most cases. Only 3 of the 13 trials had a group design which in some way tested the GFD in isolation; one of these reported improvements in outcomes [85]. Others typically focus on the GCFD (the most investigated of all interventions), while some use unique interventions such as Grimaldi et al. [82] who primarily tested a probiotic mixture (in combination with the GCFD). The study by Adams et al. [83] is also notable for the approach of sequentially accumulating interventions over a year which included the likes of dietary supplementation and epsom salt baths, with the GCFD being added at day 210.

The majority of studies are also unblinded (8 of 13) which raises a risk of placebo/nocebo effects. Some trials which are blinded use as a placebo gluten-free versions of food (bread etc.) given to participants on the assumption that they will not be able to tell the difference, meaning that a degree of skepticism is warranted even for those with such an experimental

design. Authors of non-blinded trials that achieve significant results acknowledge this limitation but highlight the practical difficulty of effective blinding for a GFD over a long period of time, or blinding of the other mixed interventions employed. This often leads to varying levels of "blindedness" within a trial, depending on the specific intervention/outcome examined. For example Adams et al. [83] write "A strength of the study is that it was a randomized, controlled study, but a major limitation of this study is that implementation of a healthy, HGCSF (healthy/gluten/casein/soy-free diet) does not allow blinding of participants. The RIAS evaluation was single-blinded, and the CARS and SAS-Pro were semi-blinded (the evaluators were blinded, the participants were not), so those results are fairly robust. The parent evaluations certainly are subject to some placebo-effect but provide an upper-bound on possible benefits. The laboratory measurements were conducted in a blinded manner, so those results should be reliable."

Studies variably do or do not use dietary "run-in" periods, which would be generally advisable to account for delays in physiological adjustment between different regimens when taking experimental measurements. Some trials are conducted over very short timeframes, such as Navarro et al. [88] which ran for 4 weeks or Pusponegoro et al. [87] which ran for 1 week. The implication of this will vary depending on the outcomes measured, but regarding gluten it is for example known that resolution of symptoms due to gluten exposure can take a number of weeks in patients with CD [92], while achieving gliadin antibody negativity can take 6 months or longer [93]. This emphasises the need for long term trials if adequate time is to be given for changes to be captured. In terms of assessing change, the measurement scales used are also scarcely replicated between studies. The majority of RCTs employ a set of tools which are unique to that particular trial, further complicating comparisons or synthesis of findings. An effort to arrive at an agreed-upon set of outcomes would benefit these trials greatly, as would purposeful replication of already-reported significant findings using the same measurement techniques. Otherwise, it also remains an open question as to how much current findings are driven by e.g., different tool sensitivities.

Taken together, it is very difficult to identify a single trial which arguably addresses all of these concerns. Ghalichi et al. [85] is the largest of those identified (80 subjects randomised), but this ran for 6 weeks and was not blinded. The longest running trials were Whiteley et al. [90] and Adams et al. [83], which took principle measurements over 12 months. Each of these did have modest sample sizes (*N* = 73 and *N* = 67 randomised, respectively), but neither were blinded and as discussed Adams et al. included a wide range of accumulative interventions. This highlights a real gap, wherein a well-powered, long-duration and placebo-controlled trial of either the GFD or GCFD has not yet been conducted. Such an experiment would ideally be run after a community consensus is reached regarding what outcomes should be focused on. Until such a trial is conducted a confident, overall conclusion cannot be made. Currently therefore, the overall pattern of the available literature does not support a proved benefit of the GFD in people with ASD (who do not have a clinical diagnosis of CD).

7. Adoption of the GFD and GCFD in ASD

Regardless of there being inconclusive evidence of a benefit to the GFD or GCFD in ASD, adoption of speciality diets is high. Studies assessing this also frequently attempt assessment of possible benefits of the diet primarily via cross-sectional analyses utilising symptom scales/survey responses, or anecdotal reporting from caregivers.

Bowers [94] reported that a majority of ASD referrals to their diet service regarded a suggestion to go on a GCFD (54.1%). Two of these 14 referrals later saw families of the patient report a "transformation" following adoption of the GFD diet ("One family described a 90% improvement and another family described an 'awakening' from a different level of consciousness"). A small comparison study [95] of children with ASD who were and were not following the GCFD reported that 7 of 13 children with ASD were already on a GCFD when recruited (outcome measures did not differ significantly from the 6 of 13 who were

not on the diet, however parents of all children on the GCFD reported that it had improved symptoms and behaviour). Babinska et al. [20] found 20.7% of children and adolescents with ASD to follow a diet which in some way restricted gluten (either GFD or GCFD); it was not found that the following of speciality diet correlated with GI symptom severity. Another study [96] found that 12% of their cohort of children with ASD consumed a GCFD, with these children also more likely to take supplements and overall showing better intake of nutrients including vitamin E, D and magnesium.

Hopf et al. [97] surveyed parents of children with ASD to identify reasons why they engaged with "complimentary and alternative medicine" (CAM). The GCFD had been used at some point by 54.8% of responders, although this was not rated among the interventions which were perceived as having had the greatest effectiveness (which included sensory integration therapy, melatonin and prescription antifungal medication). A similar study [98] also focused on the use of CAM in children with ASD as measured by caregiver report. Here, the GFD was followed at a lower rate (10% of the whole group), but was still the most common speciality diet followed. Rubenstein et al. [99] found 20.4% of children with ASD had ever used a GFD; those currently engaging with it had started on the suggestion of a medical professional in 50.7% of cases. Self-reported (from caregivers) data which predicted use of the GFD included GI conditions and developmental regression. Another experiment [100] examined all inpatients at a university medical centre, who did not have CD but who followed a GFD, to find predictors as to why in terms of comorbidities. In this, it was observed that having ASD led to an odds ratio of being on the diet of 23.42; by far the highest of all significant conditions reported (the next being irritable bowel syndrome with an OR of 6.16).

Studies which focus more directly on matching parental reporting of dietary practice to behavioural outcomes include Pennesi et al. [101]. Here, reports from 387 parents/caregivers of children with ASD were examined which focused on GI symptoms, suspected food sensitivities and adoption of speciality diets (primarily GCFD). Within these reports statistical effects were noted wherein greater suspicion of GI problems predicted greater improvement in ASD outcomes following adoption of speciality diets. Strict diet engagement was also observed to be significantly related to better outcomes. Another study [102] found no associations between dietary intake (which included measurement of gluten) and GI symptoms. However, these authors compared intake of gluten in grams against study outcome and a critical observation may be that gluten often needs to be eliminated entirely to usually see any benefit.

Some research has also focused more on the motivation in parents of children with ASD to adopt a GFD or GCFD. Marsden et al. [103] noted that parents who adopted these diets for their children with ASD were most influenced by "anticipated regret, positive outcomes and attitude". Perceived control was also relevant as a factor (with more predicting use of the diet). Tarnowska et al. [104] also investigated a similar question of what influenced parents of children with ASD to purchase GCFD foods. Packing features such as clear labelling that the food was e.g., gluten-free made them more likely to buy, while social issues around following exclusion diets (e.g., going out for a meal) and the expense/limited range of GCFD foods were seen as negative points. A survey study [105] found that approximately three quarters of clinical professionals who care for people with ASD had been asked at some point about the GCFD, while 29.5% of parents reported use of the GCFD specifically. Inadequacies with the knowledgebase regarding the use of speciality diets were noted by respondents.

Adoption of the GFD or GCFD in children with ASD is therefore quite pronounced, with lower estimates starting at 10%, and multiple studies reporting >50%. Motivations to engage with the diet appear to revolve around anticipated regret of negative outcomes should it not be tried, as well as a parent having a higher degree of perceived control. A recurring theme in a number of studies is the anecdotal reporting (e.g., by parents) of improvement in ASD outcomes, which are often isolated in incidence but apparently

dramatic in effect. Caregivers and clinicians each highlight that greater understanding of how these diets interact with ASD is required.

8. Nutritional Considerations

Limited research has also studied the impact of the GFD or GCFD on the nutritional health of children with ASD. Studies which indicate a positive consequence of following the GFD/GCFD on health include one by Herndon et al. [106]. While this focused on comparisons between (all) children with ASD compared to TD children, a subgroup analysis revealed those with ASD who followed a GCFD had higher vitamin E intake than those who did not. As already discussed, Stewart et al. [96] found following a GCFD led to higher levels of vitamin D, E and magnesium, possibly relating to a higher likelihood of simultaneously using supplements compared to those following a regular diet. Supporting this, another study [107] found that those on a GCFD were far more likely to take vitamin D and calcium supplements; no child who followed the GCFD had a deficiency of 25(OH)D (a marker of bone health), compared to 24% of those on a regular diet who did.

Studies of no or mixed outcomes include one [108] where no difference was found in nutritional intake between children with ASD who did and did not follow a GCFD (although an overall effect of suboptimal intake was noted across the whole cohort). Analysing food diaries, Mari-Bauset et al. [109] also reported mixed outcomes wherein children who followed a GCFD had lower BMI and energy, and lower intake of some nutrients (sodium, calcium, phosphorus and pantothenic acid). Conversely however, they had better intake of fiber, legumes, vegetables and fat.

Studies reporting negative impacts of the diet include Arnold et al. [110], who found a trend for children with ASD who followed the GCFD to have more deficiencies relating to essential amino acids, including tryptophan. This was replicated by another investigation [111] which also detected lower tryptophan in children with ASD compared to TD controls (it was lowest in those with ASD who followed a restricted diet). These authors hypothesised this may lead to a worsening of ASD symptoms.

9. Synthesis of Literature and Future Directions for Research

The interest of speciality diets in ASD, and particularly the GCFD, has increased markedly in the last 2 decades both in terms of adoption in the ASD community as well as scientific study. Research does convincingly demonstrate certain effects and associations which justify this. Most notably is a modest comorbidity between CD and ASD. Also shown is physiological evidence of inadequate digestion of gluten in people with ASD, leading to elevated "exorphins"/gluten antibodies around which reasonable hypotheses exist regarding downstream negative consequences on the central nervous system. Whether these associations are because of a unique relationship between CD and ASD or because of a predisposition in people with ASD to have a generally higher rate of autoimmune-like features, is yet to be resolved. Further research which directly examines the effects of these gluten products is needed in people with ASD, while additional studies examining shared genetic predispositions would also be warranted and beneficial. There is also a scarcity of epidemiological research characterising the comorbidity of ASD and CD; while one very well powered study does exist and supports this to be the case replication elsewhere is desirable. The availability of newer gluten-related antibodies (e.g., TG6) and the use of native antigliadin antibodies that are known to be sensitive to the whole spectrum of gluten-related disorders, may provide a good opportunity for further large scale epidemiological studies.

Arguably the greatest gap in current literature relates to the lack of tightly designed trials of the GFD, or GCFD in people with ASD. Those that are currently available suffer from very pronounced heterogeneity regarding the intervention followed, sample size, trial duration, blinding and outcomes measured. There does not yet exist an RCT which combines an at-least modest sample size with a placebo-controlled design over a long duration. This would be highly valuable to address, accepting the limitations of the

difficulties of such an intervention (gluten free diet) in the context of what is a behaviourally-complex cohort of patients.

Adoption of the GFD and GCFD appears to be very high amongst people with ASD. An impression is gained of strong anecdotal evidence of a benefit in relevant studies, although statistical associations do not as often bear this out. It is also unclear if any reported behavioural benefits would be because of a direct interaction between physiological gluten/casein-related impacts on the brain, or if engaging with speciality diets simply reduce non-specific GI symptoms and thus improve quality of life in a more general sense.

The nutritional impact of a GCFD on the child with ASD generally seems to be slight, or even associated with improved intake. However, some studies showing deficiencies of certain nutrients highlight the need to still maintain a balanced diet once on a restricted one. Finally, a general observation is the abundance of research which focuses on children. Of the initial papers found in the literature review, 68 (of 79) studied groups which were exclusively children, or mixed children and adolescents. While this is likely an outcome of opportunity and convenience sampling effects, it is nonetheless a strong bias within the available literature and means that generalisation of anything discussed in this review to adults with ASD is difficult.

10. Conclusions

This review highlights a modest comorbidity between ASD and CD, and a base of evidence on which reasonable hypotheses may be built to explore if gluten has a generally adverse effect in exacerbating the symptoms and quality of life in children with ASD. However, a negative effect of gluten ingestion in ASD has not been proved. Trials which have sought to demonstrate this are variable in their findings, and suffer from issues with experimental design and execution which means that an overall interpretation cannot yet be made. Efforts should focus on future studies which address limitations detailed here to create an RCT from which confident conclusions can be drawn. As diets which restrict gluten see a very high adoption rate among people with ASD, such further research is certainly warranted.

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Article

Stiff Person Syndrome and Gluten Sensitivity

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Abstract: Stiff person syndrome (SPS) is a rare autoimmune disease characterised by axial stiffness and episodic painful spasms. It is associated with additional autoimmune diseases and cerebellar ataxia. Most patients with SPS have high levels of glutamic acid decarboxylase (GAD) antibodies. The aetiology of SPS remains unclear but autoimmunity is thought to play a major part. We have previously demonstrated overlap between anti-GAD ataxia and gluten sensitivity. We have also demonstrated the beneficial effect of a gluten-free diet (GFD) in patients with anti-GAD ataxia. Here, we describe our experience in the management of 20 patients with SPS. The mean age at symptom onset was 52 years. Additional autoimmune diseases were seen in 15/20. Nineteen of the 20 patients had serological evidence of gluten sensitivity and 6 had coeliac disease. Fourteen of the 15 patients who had brain imaging had evidence of cerebellar involvement. Twelve patients improved on GFD and in seven GFD alone was the only treatment required long term. Twelve patients had immunosuppression but only three remained on such medication. Gluten sensitivity plays an important part in the pathogenesis of SPS and GFD is an effective therapeutic intervention.

Keywords: stiff person syndrome; anti-GAD antibodies; gluten sensitivity; coeliac disease; cerebellar ataxia; gluten free diet

1. Introduction

Glutamic acid decarboxylase (GAD) is the enzyme involved in the synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). GAD is found in both the central and peripheral nervous systems and in pancreatic beta cells [1]. GAD antibodies were first detected and characterised in children with newly diagnosed insulin dependent diabetes mellitus (IDDM) [2]. These were shown to be reacting with pancreatic islet cell proteins.

The first neurological disease to be associated with anti-GAD antibodies was stiffperson syndrome (SPS) [3]. SPS is a very rare autoimmune neurological disease, clinically characterised by axial rigidity, often resulting in hyperlordosis, painful spasms and anxiety. It belongs to a spectrum of CNS hyperexcitability syndromes. SPS is often associated with additional autoimmune diseases such as hypothyroidism, IDDM, pernicious anaemia and others. The majority of patients with SPS have anti-GAD antibodies. Anti-GAD antibodies have also been found in some cases of sporadic idiopathic ataxias [4]. Their presence implies an autoimmune pathogenesis raising the possibility of therapeutic interventions with immunosuppressive medication.

We have previously made a connection between anti-GAD associated diseases and gluten sensitivity (GS) including coeliac disease (CD) [5]. We were also able to show considerable overlap between anti-GAD ataxia and gluten ataxia (70% of patients with anti-GAD ataxia are gluten sensitive), and we have demonstrated that gluten free diet

(GFD) is an effective therapeutic intervention in such patients [6]. In this report we share our experience in managing and treating patients with SPS and in particular highlighting the overlap between SPS, gluten sensitivity and CD as well as reporting the therapeutic effect of gluten free diet (GFD).

2. Methods

This report is based on a retrospective observational case series of patients regularly attending our specialist clinics (GS/neurology, neuroimmunology and ataxia). The South Yorkshire Research Ethics Committee has confirmed that no ethical approval is indicated given that all investigations/interventions were clinically indicated and did not form part of a research study. All patients were identified from these clinics by one of the authors (MH) who is in charge of the clinical care of all these patients. The patients have been looked after for over 25 years and are under regular follow up by the same consultant neurologist. The diagnosis of SPS was based on the typical clinical features (stiffness, axial rigidity, episodic painful spasms) in addition to the presence of high titre of anti-GAD antibodies (>2000 U/mL) and neurophysiological evidence of CNS hyperexcitability (continuous motor unit activity on EMG and/or abnormal blink reflex).

Serological testing in addition to anti-GAD antibodies, included antigliadin antibodies (AGA, Phadia), TG2 (Phadia), endomysium antibodies (EMA, Werfen) and TG6 antibodies (Zedira). Those patients with one or more positive antibodies were offered gastroscopy and duodenal biopsy to establish the presence of enteropathy (triad of villous atrophy, crypt hyperplasia, increased intraepithelial lymphocytes). All patients with positive serology for gluten sensitivity were advised to adopt a GFD irrespective of the presence of enteropathy. They were all reviewed by an experienced dietitian and given detail advice on GFD. Depending on clinical response after GFD some patients were also offered treatment with immunosuppression. This included intravenous immunoglobulins, azathioprine, mycophenolate, rituximab, plasma exchange and cyclophosphamide.

All patients underwent brain imaging with MRI, some also had MR spectroscopy of the cerebellum. Cerebellar involvement in the context of SPS is almost universal but often under-reported by patients because their most disabling symptoms are those of stiffness and painful spasms.

3. Results

We identified 20 patients with SPS over the last 25 years. There were 11 female and 9 male patients. Mean age at onset of symptoms was 52 (range 37–69 years). The presenting symptoms included primarily leg stiffness in 12, truncal stiffness in 5, painful spasms in 2 (painful spasms became a prominent feature in most patients later on in the disease), ataxia in 2 (later a feature in 17 patients) and one leg stiffness in one. Additional autoimmune diseases (apart from GS and CD) were present in 15 patients with 11 having hypothyroidism, 7 having IDDM, 2 having myasthenia gravis, 2 having Sjogren's syndrome, 1 pernicious anaemia and 1 psoriatic arthropathy (some patients had more than one autoimmune disease). Serological evidence of GS (one or more of AGA, EMA, TG2 and TG6 antibodies) were found in 19 of the 20 patients (95%). The diagnosis of gluten sensitivity was made in Sheffield by the authors and only one of the patients had a pre-existing diagnosis of CD. Fourteen patients underwent gastroscopy and duodenal biopsy. Six patients had evidence of CD on biopsy, the remaining 8 had a normal mucosa. Only 3 of the 19 patients with GS/CD had any gastrointestinal symptoms (diarrhoea, bloating, abdominal pain) attributed to GS/CD. All of these 3 had coeliac disease on biopsy and in all 3 the gastrointestinal symptoms improved on a GFD. The above results are summarised in Table 1.

Table 1. Clinical characteristics, investigations and outcomes of 20 patients with stiff person syndrome.

number of patients with SPS reported	20
male: female	9:11
mean age at symptom onset (range)	52 (37–69 years)
additional autoimmune diseases	11 hypothyroidism, 7 IDDM, 2 myasthenia gravis, 2 Sjogren's, 1 pernicious anaemia, 1 psoriatic arthropathy
serological evidence of gluten sensitivity (patients may have been positive for more than one antibody)	19/20 (95%) (17 AGA, 7 TG6, 5 TG2, 5 EMA)
coeliac disease (out of 14 patients who had duodenal biopsy)	6
abnormal neurophysiology showing continuous motor unit activity	13, normal in 4, not done in 3
abnormal blink reflex	abnormal in 2, only done in 4
abnormal MR spectroscopy of the cerebellum suggestive of cerebellar involvement	14/15 (93%)
Improved on gluten free diet	12/19 (63%)
number that tried immunosuppression (still on immunosuppression)	12 (3)

Please note that not all patients were tested for TG6 antibodies. (IDDM-insulin dependent diabetes mellitus, AGA-antigliadin antibodies, TG6- transglutaminase 6 antibodies, TG2-transglutaminase 2 antibodies, EMA-endomysium antibodies).

Neurophysiology showed continuous motor unit activity in keeping with SPS in 13 of the patients. Three patients did not have neurophysiology but the clinical phenotype in combination of high anti-GAD was sufficient to enable the diagnosis. The remaining 4 patients had normal neurophysiology, but this was done after the patients were established on antispasmodic medication. Abnormal blink reflex was seen in 2 patients, but this was only performed in 4 patients.

GFD was recommended to all 19 patients with gluten sensitivity. In 12/19 patients (5 with CD and 7 with GS and no enteropathy) the GFD was found to be beneficial to their SPS and ataxia symptoms (reduced frequency of spasms, stabilisation of mobility, rigidity and improved ataxia). Five patients did not derive any benefit. Two patients did not go on GFD. In 7 patients (3 with CD and 4 with GS and no enteropathy) GFD alone was the only treatment needed to keep their symptoms under control and stabilise their condition. Immunosuppression was used in 12 patients. This included intravenous immunoglobulins (IVIgs) in 9, mycophenolate in 5, plasma exchange in 2, azathioprine in 1, rituximab in 1 and cyclophosphamide in 1 (some patients had more than one immunosuppressive medication). One patient underwent autologous stem cell transplantation because nothing else was effective. This resulted in stabilisation [7]. Only one of the 9 patients that received immunoglobulins found it beneficial long term. In the remaining, oral immunosuppression was felt to be more effective than repeat IVIg's. All apart from 3 patients were taking antispasmodic medication. Six on 3 different antispasmodics, 5 on 2 and 6 on one. The most commonly used antispasmodics were baclofen (14), dantrolene (9), diazepam (8) and tizanidine (1). One patient with very resistant disease also had botox injections and found entonox very helpful for the painful spasms. Ten patients were on Gabapentin. The addition of this medication seemed to offer some additional benefit in partially controlling/stabilising their symptoms.

MR spectroscopy of the cerebellum was done in 15 patients. Evidence of reduced NAA/Cr ratio in the vermis and/or hemisphere was seen in 14 (mean NAA/Cr from the vermis was 0.9 (range 0.79–1.12) and from the hemisphere 0.95 (range 0.74 to 1.1), normal ratio should be above 1, in keeping with cerebellar dysfunction. Only one of the 15 patients had normal spectroscopy (NAA/Cr vermis 1.03 and hemisphere 1.23). Follow up MR

spectroscopy was available in 10 patients who were also GS (5) or had CD (5), after staring GFD (no immunosuppression). Eight showed improved MR spectroscopy (NAA/Cr are ratio) from the vermis whilst 2 showed deterioration.

At the time of writing this report 5 patients had died. Three as a result of SPS (2 in hospital with complications of immobility, one had respiratory arrest resulting in brain hypoxia during a severe chest spasm causing respiratory arrest). One died of myocardial infarction (also had IDDM) and the other due to COVID pneumonia. Of the remaining 15 patients 11 remain mobile with minimal walking aids (use of one stick) and 4 are wheel-chair bound.

Out of the 15 patients who are still alive, 12 are still on a strict GFD, and only 3 are on immunosuppression (1 regular IVIgs, 2 on mycophenolate).

Illustrative Clinical Case

This 60-year-old man was referred to our centre 4 years ago with an established diagnosis of SPS. He had received high dose steroids on several occasions after being admitted with painful spasms that rendered him bed bound. The episodes of severe spasms had become so disabling that the patient had become understandably anxious and fearful of leaving the house.

At the time of the initial assessment, he was on no regular immunosuppression, but the referring neurologist was planning the introduction of IVIgs. On clinical examination in addition to severe stiffness in both legs and exaggerated lordosis he had evidence of incoordination with nystagmus on lateral gaze, finger nose and heel to sheen ataxia. He also had gait ataxia. He was using a wheelchair when out of his home.

He had a history of hypothyroidism. Investigations showed high titre of anti-GAD antibodies (>2000 U/mL), with neurophysiological evidence of continuous motor unit activity on EMG which, in combination with the clinical presentation led to the diagnosis of SPS.

In addition to the high levels of anti-GAD antibodies he was positive for AGA, EMA, TG2 and TG6 antibodies. Duodenal biopsy confirmed the presence of CD. MR spectroscopy confirmed abnormal NAA/Cr ratio over the vermis in keeping with the clinical findings of gait ataxia (Figure 1). He started on a strict GFD. Within the first 6 months he observed significant reduction in the painful spasms. This was without any other medication. His mobility improved and he was now able to walk using a frame and also go out of his home. After a year on GFD his gluten sensitivity-related antibodies were no longer present. Repeat MR spectroscopy of the cerebellar vermis showed improved NAA/Cr ratio (from 0.84 to 0.90, normal ratio should be over 1) reflecting the improved mobility. He is still stable (3 years after the introduction of GFD) and not requiring any immunosuppression.
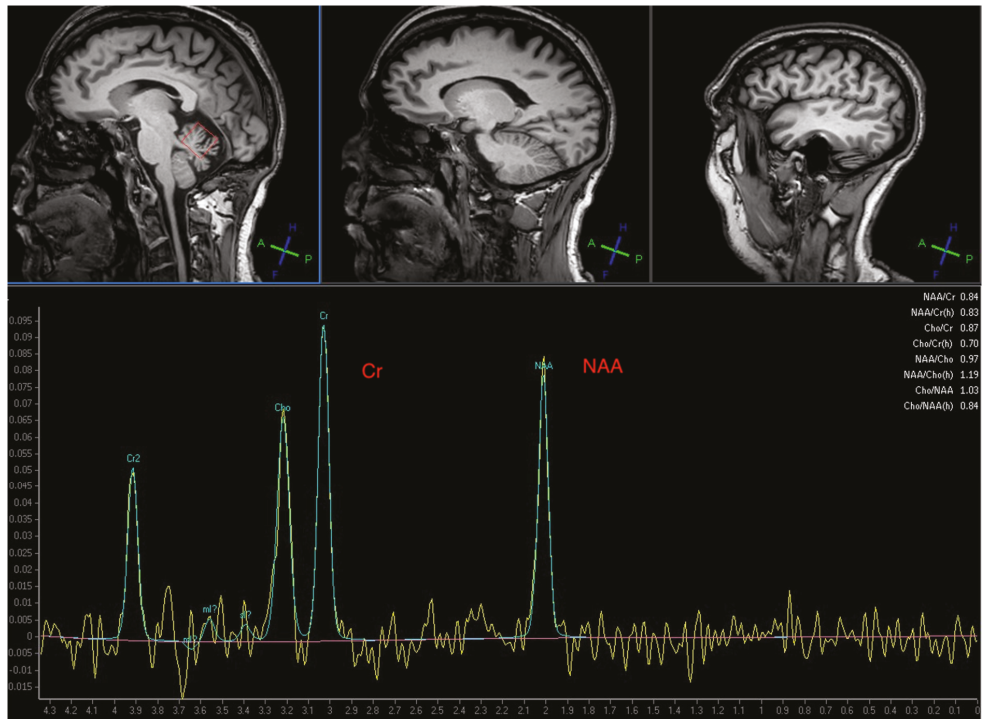
Figure 1. Magnetic Resonance Spectroscopy of the cerebellar vermis from the illustrative clinical case (see text) showing a significant reduction of the N-Acetyl-Aspartate to Creatine ratio (NAA/Cr) at 0.84 (normal should be above 1). All but one of these patients with stiff person syndrome (SPS) had abnormal spectroscopy of the cerebellum highlighting the fact that cerebellar involvement is universal in SPS.

4. Discussion

We have previously reported an association between anti-GAD related diseases and GS/CD [5]. We have also recently published our experience in the management of 50 patients with anti-GAD ataxia where we have again shown a significant overlap between anti-GAD ataxia and gluten ataxia (70% of patients with anti-GAD ataxia were gluten sensitive) [6]. Furthermore, we have shown that patients with anti-GAD ataxia who are gluten sensitive respond well to strict GFD, with improvement of the ataxia. In this report we present our experience of managing 20 patients with SPS, primarily to highlight the strong association with gluten GS/CD (95% positive for one or more gluten sensitivity-related antibodies and at least 30% having CD) and demonstrate that GFD has a therapeutic role to play. The prevalence of AGA antibodies in the healthy population was 12% and that of CD 1% [8].

The association between SPS and gluten sensitivity cannot be simply explained on the basis of an association of two autoimmune diseases by chance. Nineteen of these 20 patients (95%) with SPS were found to be gluten sensitive. In addition, we have shown that GFD has an important therapeutic role to play in these patients, suggesting that GS/CD must play a role in the pathogenesis of SPS.

As per the case highlighted in this report, the majority of patients who are on GFD have found this intervention helpful in controlling their SPS symptoms.

In our experience, cerebellar involvement in the context of SPS appears to be universal in these patients. In fact, cerebellar ataxia in isolation is a commoner manifestation of

anti-GAD related diseases than SPS based on our experience; the number of patients with anti-GAD ataxia we have treated is 50 as opposed to 20 with SPS. However, there may be some referral bias given that our unit is one of the National Ataxia Centres in the UK.

Cerebellar involvement in the context of SPS may have an important pathophysiological role to play; the output of the cerebellum is all inhibitory. Any dysregulation of such output could potentially result in a state of CNS hyperexcitability. This state of hyperexcitability is particularly prominent in the immune ataxias by contrast to the genetic or degenerative ataxias [9]. It can manifest with rigidity and spasms, as is the case in SPS but also with cortical myoclonus as is often seen in cases of refractory CD [10]. Additional clinical markers of hyperexcitability include brisk reflexes and exaggerated startle. It is possible that the selective involvement of different cerebellar cell populations may explain why the state on brain hyperexcitability is more often seen in immune rather than genetic ataxias.

The association between anti-GAD antibodies and gluten sensitivity merits further consideration. Ventura et al. have made the observation that the prevalence of additional autoimmune diseases in children with CD is significantly lower than in those patients with CD diagnosed in adulthood [11]. They concluded that GFD may reduce the risk of developing additional autoimmune diseases later on in life. This observation echoes our previously observed reduction in anti-GAD antibodies in patients with anti-GAD related diseases and gluten sensitivity who go on a strict GFD [5].

The pathological role of anti-GAD antibodies in the genesis of SPS and ataxia is unclear. Since GAD65 (the GAD isoform implicated in these diseases) is intracellular and is associated with a range of neurological conditions, some have argued that anti-GAD65 antibodies have no pathogenic role to play. On the other hand, recent physiological studies in vitro and in vivo have demonstrated that binding of GAD by anti-GAD antibodies induces loss of GAD functions relating to GABA release, leading to the development of cerebellar ataxia [12]. Given these observations the question remains as to why should GFD be beneficial in those patients with gluten sensitivity and SPS. The response to GFD in those patients who are gluten sensitive suggests that gluten sensitivity may be driving the immune response that results in SPS.

There are no clear-cut evidence-based guidelines for the treatment of SPS. It has been shown that regular IVIgs can be beneficial [13]. This has not been our experience. Only 2 of the 20 patients reported here found regular IVIgs of some sustained benefit. Immunosuppression with mycophenolate has been beneficial for some patients but by far the most effective therapeutic intervention has been the GFD.

In terms of symptom relief most patients require a combination of one or often several antispasmotics of which in our experience dantrolene, baclofen and diazepam offer the best combination. We also have used gabapentin more as a "disease modifier" simply on the theoretical benefit based on its mode of action.

To conclude, 95% of our patients with SPS who have been under regular review at our centre have evidence of gluten sensitivity or CD and have benefited from a strict GFD. The diagnosis of gluten sensitivity relies on a range of antibodies and the use of EMA or TG2 antibodies alone, whilst sufficient to diagnose CD, cannot diagnose gluten sensitivity without enteropathy. This is an important consideration because GFD is beneficial for patients with SPS who are gluten sensitive irrespective of the presence of enteropathy.

Author Contributions: M.H. identified the link between gluten sensitivity and SPS and conceptualised this report as well as looked after all the patients. He produced the first draft. P.Z. and P.G.S. performed all the neurophysiology. D.S.S. performed all the gastroscopies and biopsies. N.H. was responsible for the imaging. All authors reviewed and contributed to the final version of the paper. All authors have read and agreed to the published version of the manuscript.

South Yorkshire Research Ethics Committee confirmed that no ethical approval is indicated given that all investigations were clinically indicated and did not form part of a research study.

Informed Consent Statement: The South Yorkshire Research Ethics Committee has confirmed that no ethical approval is indicated given that all investigations/interventions were clinically indicated and did not form part of a research study.

Data Availability Statement: Anonymised data can be provided on request.

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

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Article

Intracortical and Intercortical Motor Disinhibition to Transcranial Magnetic Stimulation in Newly Diagnosed Celiac Disease Patients

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Abstract: Background: Celiac disease (CD) may present or be complicated by neurological and neuropsychiatric manifestations. Transcranial magnetic stimulation (TMS) probes brain excitability non-invasively, also preclinically. We previously demonstrated an intracortical motor disinhibition and hyperfacilitation in de novo CD patients, which revert back after a long-term gluten-free diet (GFD). In this cross-sectional study, we explored the interhemispheric excitability by transcallosal inhibition, which has never been investigated in CD. Methods: A total of 15 right-handed de novo, neurologically asymptomatic, CD patients and 15 age-matched healthy controls were screened for cognitive and depressive symptoms to the Montreal Cognitive Assessment (MoCA) and the 17-item Hamilton Depression Rating Scale (HDRS), respectively. TMS consisted of resting motor threshold, amplitude, latency, and duration of the motor evoked potentials, duration and latency of the contralateral silent period (cSP). Transcallosal inhibition was evaluated as duration and latency of the ipsilateral silent period (iSP). Results: MoCA and HDRS scored significantly worse in patients. The iSP and cSP were significantly shorter in duration in patients, with a positive correlation between the MoCA and iSP. Conclusions: An intracortical and interhemispheric motor disinhibition was observed in CD, suggesting the involvement of GABA-mediated cortical and callosal circuitries. Further studies correlating clinical, TMS, and neuroimaging data are needed.

Keywords: gluten-related pathology; cortical excitability; transcallosal inhibition; transcranial magnetic stimulation; executive dysfunction; gamma-amino-butyric acid

1. Introduction

Within the wide spectrum of gluten-related disorders [1], it is now established that the classic celiac disease (CD) is only the tip of the "CD iceberg" [2], since from five to six-fold more subjects exhibit non-typical phenotypes [3]. As such, CD is currently viewed as a multiorgan disease with multifactorial pathogenesis and clinical manifestations.

Among extraintestinal features, neurological and neuropsychiatric manifestations are still a diagnostic challenge in CD, given that they can precede or follow the disorder or be already evident at the onset [1,4–6]. In a recent cohort prospective investigation of newly diagnosed subjects of CD [7], neurological deficits were common, and a significant volume decrease in some cerebral regions, with transglutaminase (TG)-6 autoantibodies, was observed. These findings highlight the importance of prompt diagnosis, awareness among physicians, and compliance to an adherent gluten-free diet (GFD) to prevent, or at least limit, the neurological involvement and related disability [7]. It has been also demonstrated that most of the subjects with confirmed CD referred for neurological consultation already show changes at brain magnetic resonance imaging (MRI) [8]. Based on these considerations, a reliable diagnostic tool, able to early detect process, progression, and complications underlying the disease, as well as the response to the GFD, is needed.

Within the neurophysiological techniques, motor evoked potentials (MEPs) to transcranial magnetic stimulation (TMS) are among the electrophysiological methods that can non-invasively probe the state of excitation of cortical motor areas in vivo [9] and the conduction along the cortico-spinal pathway [10], as well as the functional connectivity across hemispheres [11]. TMS is also able to unveil preclinical motor impairment in several neurological, psychiatric, and some secondary diseases of the central nervous system (CNS), also providing prognostic [12] and therapeutic implications [13]. Finally, the "pharmaco-TMS" may distinctively explore various transmission pathways within the CNS, such as that mediated by gamma-aminobutyric-acid (GABA), glutamate, acetylcholine, and monoamines, by administering drug agonists or antagonists [14–16].

To date, only a few studies have applied TMS in CD. In 1999, Pellecchia and colleagues first reported a decreased MEP size in the rectus femoris muscle in a CD patient, who improved after the GFD [17]. A year later, a delayed MEP in the left tibialis anterior muscle and a change of cortical inhibition was reported in one of three CD subjects with cortical myoclonus [18]. More recently, systematic studies before and after GFD have specifically evaluated the TMS profile of cortical excitability in CD.

In the first study, twenty de novo subjects without clinical involvement of the CNS and twenty controls matched for age were included [19]. TMS showed a hyperfacilitation and a disinhibition of the primary motor cortex (M1) in patients, suggesting an impaired glutamatergic and GABAergic circuitry, respectively. Unbalanced inhibitory and excitatory transmissions within the M1 was hypothesized as the correlate of a cross-interaction between some neuronal antigens and gliadin antibodies. Alternatively, the deposition of tissue TG-immunoglobulin might lead to a pathological ion concentration at the level of neuronal membranes. Similarly, the CNS-produced antibodies against glutamic acid decarboxylase may impair the activity of GABAergic interneurons [19].

The same sample was re-assessed following a relatively short-term GFD (median 16 months) [20]. Gastrointestinal manifestations improved although, unexpectedly, the excitation state of their M1 to TMS enhanced further. This result was thought to be an index of an adaptive re-modeling of the motor areas, probably not related to the GFD. It is also reasonable to hypothesize that the duration of the diet or its adherence was not enough to produce a significant recovery [20]. A further study following a substantially longer gluten restriction (mean period 8.35 years) revealed that only a sustained GFD could restore the TMS-associated modifications in adults with CD. However, some excitatory changes seems to persist, likely indicating a synaptic intracortical rearrangement of the "celiac brain", mostly involving the glutamate-mediated interneurons [21].

Beside the intracortical excitability, to date, no data are available on the intercortical excitability in CD. Namely, the interhemispheric motor functioning to TMS, as indexed by specific measures of transcallosal inhibition, has never been investigated in these patients. In this cross-sectional study, we aim to evaluate the callosal function to TMS in de novo, neurologically asymptomatic, CD patients compared to healthy controls. We hypothesized that these subjects, as already observed for the measures of intracortical excitability, might exhibit changes in intercortical excitability, even at a subclinical level.

2. Materials and Methods

2.1. Participants and Evaluation

Fifteen consecutive de novo subjects with CD (13 women; mean age ± standard deviation (SD): 34.10 ± 12.03 years), diagnosed according to the European Society for Pediatric Gastroenterology Hepatology and Nutrition guidelines [22], were enrolled from the Regional Center for Celiac Disease of the Azienda Ospedaliero-Universitaria "Policlinico G. Rodolico-San Marco" of Catania (Italy). Fifteen healthy individuals (12 women; mean age ± SD: 34.90 ± 9.18 years), matched for age with patients, served as the control group. All patients were on free diet at the time of the enrolment.

Criteria of exclusion were: age < 18 years; CNS (i.e., Parkinson's disease, stroke, dementia, traumatic brain injury, multiple sclerosis (MS), epilepsy, etc.) or psychiatric diseases (major depressive disorder (MDD), bipolar disorders, schizophrenia, obsessive–compulsive disorders, etc.); chronic, acute, or uncompensated medical conditions (i.e., heart failure, coronary heart disease, liver or kidney failure, etc.); illicit drug abuse or alcohol dependency; intake of drugs influencing mood or M1 excitation state (i.e., antidepressants, benzodiazepines, mood stabilizers, neuroleptics); pacemaker, pregnancy, or other conditions precluding MEP, according to the latest guidelines on TMS safety [23].

The clinical-demographic assessment consisted of: age, sex, educational level, handedness, general and neurological exams, co-morbidities. A screening test of global cognitive status by means of the Montreal Cognitive Assessment (MoCA), adjusted for age and educational level for each individual [24], and a symptom estimation of depression through the 17-item Hamilton Depression Rating Scale (HDRS) [25] were performed by an operator (M.C.) blind to the participant status as patient or control. Additionally, a computed tomography (CT) of the brain was acquired in all patients with a helical 64-slice General Electric scanner, with 2.5 mm slice thickness, in order to properly detect intracranial calcifications (that can be found in CD) and to exclude clear neuroradiological lesions.

The Ethics Committee of the Azienda Ospedaliero-Universitaria "Policlinico G. Rodolico-San Marco" of Catania (Italy) approved the study (code of approval: Prot. n.103/694). Informed consent was signed by each individual prior to participation in accordance with the Declaration of Helsinki in 1964 and subsequent amendments. Every procedure was carried out in a dedicated laboratory by experienced operators.

2.2. TMS Procedures

TMS was carried out by means of a high-power Magstim 200 stimulator (Magstim Co., Whitland, Dyfed, UK). A 70 mm figure-of-eight coil was positioned on the M1 of the dominant hemisphere at the best position of the scalp to evoke MEPs in the first dorsal interosseous (FDI) muscle of the contralateral side, according to the Edinburgh Handedness Inventory (EHI) [26]. Electromyography (EMG) was performed with silver/silver-chloride disposable self-conductive and self-adhesive surface electrodes. The active electrode was positioned on the belly of the target muscle (FDI), the reference at the metacarpal–phalangeal joint of the index finger, whereas the ground on the wrist dorsal surface. For the conduction study of the motor nerve, i.e., compound motor action potential (CMAP) and F-waves of the ulnar nerve, a bipolar nerve stimulation electrode, with an interelectrode separation of 25 mm and 6-mm diameter felt pads, was used while recording from the target muscle (FDI).

The resting motor threshold (rMT) was considered as the minimum intensity of stimulation capable to induce, at rest, a MEP of an amplitude >50 μV in five of ten trials, as recommended by the international guidelines [27]. The central motor conduction time (CMCT) was estimated by subtracting the time of conduction along peripheral nerves, calculated with the F-wave technique, from the MEP latency recorded during moderate muscular contraction, with an intensity of stimulation of 130% with respect of the rMT. F-waves and peripheral CMAP were evoked with electrical supramaximal stimulations of the right ulnar nerve at wrist. The MEP size was measured as a percentage of supramaximal CMAP size (i.e., the amplitude ratio), which provides a more reliable estimation than the

peak-to-peak MEP size [27]. The MEP duration (in ms) was measured from the latency of onset to the return to baseline for both resting and facilitated MEPs [28]. As known, a prolonged MEP duration reflects a temporal dispersion of the cortico-spinal response, thus suggesting a CNS pathology affecting the central motor pathway [27].

The assessment of silent periods (SPs), i.e., contralateral SP (cSP) and ipsilateral SP (iSP), represents the main single-pulse TMS methods for exploring and quantifying the intracortical and intercortical motor inhibitory components, respectively [29]. Moreover, as SPs reflect an index of inhibition of volitional motor activity, rather than a MEP inhibition per se, they are of particular interest for exploring the inhibitory components of the cortico-spinal tract and the interhemispheric correlates of voluntary motor output [27].

When TMS is applied to the M1 contralaterally to the target muscle, the obtained parameter is named cSP [29]. In this case, TMS typically elicits a MEP in the target muscle, followed by a suppression of the voluntary ongoing EMG activity for a period of up to some hundred ms [30]. The cSP is then quantified by its duration, with a longer cSP interpreted as a greater cortical inhibition and shorter duration as a cortical disinhibition [29]. The cSP is generated by both cortical and spinal contribution: the first portion (0–50 ms) is considered to be of spinal origin [30,31], including after-hyperpolarization of motor neurons and recurrent inhibition by the activation of Renshaw cells, or double synaptic inhibition via the Ia inhibitory interneurons [30–34]; the later part (50–200 ms) is attributed to an intracortical inhibition of the cortico-spinal output [30,31,35–37]. Since the contribution of cortical mechanisms is considered to be larger (75%) than the spinal ones (25%), the cSP is assumed to reflect the activation of intracortical inhibitory interneurons, mainly by the GABAergic transmission within the M1, particularly by the GABA-B receptors [38,39].

The iSP is evoked by applying TMS to the same hemisphere of a tonically contracting muscle, and, as such, it is viewed as the correlate of transcallosal inhibition [40]. Proposed mechanisms are the following: TMS pulses activate glutamatergic (excitatory) callosal motor fibers synapsing on GABAergic (inhibitory) interneurons in contralateral M1 [41,42]. This would cause a net inhibitory effect and result in a brief depression of the descending cortico-spinal activity that supports the tonic muscle contraction [41,42]. In the contracting muscle, this will appear as a brief suppression or attenuation of the ongoing EMG activity. As for the cSP, the iSP is also quantified by its onset and duration, with greater duration interpreted as a more intense interhemispheric inhibition, and vice versa [29]. Unlike the cSP, the iSP is assumed to be a fully cortical phenomenon: indeed, the iSP does not lower the amplitude of the H-reflex, thus suggesting a lack of any spinal contribution [40].

In the present study, the cSP and iSP were recorded with ~50% of the maximal voluntary tonic contraction of the FDI, evoked by single TMS pulses at 130% of rMT, as recommended [27]. For both recordings, 10 single stimuli were delivered to the contralateral and ipsilateral M1, respectively, and a brief pause (~20 s) was allowed following each stimulus to decrease the possibility to be fatigued. The onset of the cSP and iSP (i.e., their latency) was evaluated for waveform averaged as the temporal interval where the EMG activity dropped ≤75% of the amplitude of the pre-stimulus level. The mean cSP and iSP duration of the rectified trials was considered, with duration measured for all traces as the time from when the EMG-rectified activity dropped ≤75% of the pre-stimulus level to when it returned >75%. This activity level was considered for onset and ending of the cSP and iSP, in order to obtain an objective and reproducible analysis, thus reducing the risk of doubtful interpretation and minimizing bias [29].

A standardized safety checklist was used to screen all individuals [23] and to exclude any neurological disease or medication possibly affecting CNS excitation state. All procedures were performed with participants seated in a dedicated armchair with constant EMG monitoring to guarantee a desirable level of tonic EMG activity during contraction or a total muscular relax. Once collected, data were stored on a dedicated PC by means of ad hoc software that allows one to acquire, process, and analyze data [43]. To reduce the intersubject variability, TMS recordings were executed in the same lab and experimental conditions, at the same time (~11:30 a.m.), and by the same trained operators.

2.3. Statistical Analysis

Given the non-normal distribution of most data, non-parametric statistics were adopted. The Mann–Whitney test for independent datasets was used for between group comparisons, followed by the Bonferroni correction for multiple comparisons. In order to avoid missing significant differences due to the relatively low number of individuals recruited, we also calculated the effect size of all differences between patients and controls with the rank-biserial correlation by Wendt ($r = 1 - (2U)/(n1\ n2)$) [44]. With this approach, an r of 0.1 is considered as "small", 0.3 "medium", and 0.5 "large". The Spearman's rank correlation coefficient was used to evaluate the correlations.

3. Results

Table 1 summarizes clinical-demographic and serological features, as well as data from the main diagnostic exams. The right-handedness of all participants was confirmed by the EHI. The general examination was unremarkable in all participants, with the exception of two overweight patients and one underweight patient. Apart from a patient with diffuse and symmetric brisk tendon reflex at upper limbs (without any pathological reflex, including the Hoffmann sign), neurological exams were all normal. Three subjects of the CD group had co-morbidities: autoimmune thyroiditis (one), Raynaud phenomenon (one), and fibromyalgia and psoriasis (one). All subjects were drug-free, except for a patient taking l-thyroxine, with normal levels of thyroid hormones. The two groups were comparable for age, gender, anthropometric features (height, weight, and body mass index), and educational level. Scores at MoCA and HDRS were significantly worse in the CD group than in controls, with a large effect size (Table 2), although the difference of MoCA only remained significant after the Bonferroni correction. Brain CT ruled out intracranial calcifications and clear neuroradiological abnormalities.

The cSP and iSP durations were significantly shorter in CD subjects compared to controls, with a large effect size and also after the Bonferroni correction, whereas no significant difference was found for their latency. Finally, a smaller MEP amplitude was observed in patients than controls, with a large effect size, but not after correction, and with a comparable amplitude ratio between the two groups (Table 2).

Lastly, correlations between the TMS measures and clinical data that were found to be significantly different (cSP duration, iSP duration, and MoCA) disclosed a significant positive correlation between the MoCA score and iSP duration in patients (Figure 1).

Table 1. Clinical, laboratory, and instrumental features of celiac disease patients.

No.	Age (Years)	Sex	Family History	Clinical Symptoms	Co-Morbidities	Antibodies	Endoscopy	Histopathology
1	55	F	+	Tiredness, dyspepsia, weight loss, iron deficiency anemia	-	tTG, EMA	Scalloped duodenal folds	3c
2	18	F	+	Asthenia, iron deficiency anemia	-	tTG, EMA	Scalloped duodenal folds	3c
3	25	F	+	Tiredness, iron deficiency anemia, dermatological manifestations	-	tTG, EMA	Scalloped duodenal folds	3c
4	18	F	-	Headache, tiredness, belly pain, iron deficiency anemia	-	tTG, EMA	Scalloped duodenal folds	3c
5	29	M	+	- (familial screening)	-	tTG, EMA	Scalloped duodenal folds	3c
6	45	M	-	Tiredness, weight loss, headache, iron deficiency anemia, abdominal pain	-	tTG	Scalloped duodenal folds	3c
7	36	F	-	Headache, tiredness, iron deficiency anemia, vitamin D deficiency weight loss	Autoimmune thyroiditis	tTG, EMA	Scalloped duodenal folds	3c
8	27	F	-	Abdominal pain, diarrhea, tiredness, unsteadiness, weight loss, iron deficiency anemia	-	tTG, EMA	Scalloped duodenal folds	3c
9	35	F	-	Abdominal pain, diarrhea, nausea, iron deficiency anemia, tiredness	-	tTG, EMA	Scalloped duodenal folds	3c
10	44	F	+	Iron deficiency anemia, stipsis and diarrhea, headache, tiredness	Fibromyalgia, psoriasis	tTG	Scalloped duodenal folds	3c
11	45	F	-	Diarrhea, abdominal discomfort, tiredness	Raynaud phenomenon	tTG	Moderate atrophic villi	3b
12	41	F	-	Dyspepsia, iron-deficiency anemia, diarrhea, weight loss, tiredness, diffuse pain	-	tTG, EMA	Scalloped duodenal folds	3c
13	49	F	-	Alternate alvus, dyspepsia, asthenia, tiredness	-	tTG	Scalloped duodenal folds	3c
14	24	F	-	Tiredness, dyspepsia, weight loss, iron deficiency anemia	-	tTG, EMA	Scalloped duodenal folds	3c
15	20	F	-	Tiredness, iron deficiency anemia	-	tTG, EMA	Scalloped duodenal folds	3c

Legend: F = female; M = male; tTG = tissue transglutaminase antibodies; EMA = endomysial antibodies. Classification of histopathology according to the Marsh–Oberhuber grading system [45]: 3a = mild villous flattening; 3b = severe villous flattening; 3c = complete villous flattening; + = positive/present; - = negative/absent.

Table 2. Comparison of clinical features and TMS data of both patients and controls.

Variable	Celiac Disease (n = 15) Mean ± SD	Healthy Controls (n = 15) Mean ± SD	Mann–Whitney U	p	Effect Size r
Age, years	34.10 ± 12.03	34.90 ± 9.18	102	NS	0.093
Height, m	1.60 ± 0.08	1.70 ± 0.09	70.5	NS	0.373
Weigh, Kg	57.90 ± 17.38	61.10 ± 8.31	73	NS	0.351
BMI, Kg/m²	21.80 ± 5.99	21.80 ± 2.10	80	NS	0.289
Education, years	14.60 ± 3.44	16.20 ± 3.97	69.5	NS	0.382
MoCA	25.80 ± 2.40	28.00 ± 1.00	46	**0.0062 ***	0.591
HDRS	8.30 ± 6.30	2.90 ± 2.19	50.5	0.01	0.551
rMT, %	37.10 ± 5.58	36.90 ± 6.42	109.5	NS	0.027
cSP duration, ms	87.30 ± 26.85	123.10 ± 29.71	37	**0.0019 ***	0.671
cSP latency, ms	44.70 ± 3.81	44.10 ± 3.10	104.5	NS	0.071
iSP duration, ms	20.50 ± 3.54	25.50 ± 3.32	33.5	**0.0011 ***	0.702
iSP latency, ms	32.90 ± 5.84	34.50 ± 4.80	82	NS	0.271
MEP latency, ms	20.00 ± 1.24	20.30 ± 1.56	97.5	NS	0.133
MEP duration, ms (at rest)	12.4 ± 1.42	13.4 ± 2.04	79.5	NS	0.293
MEP duration, ms (active)	15.4 ± 2.43	15.7 ± 1.62	98.5	NS	0.124
CMCT, ms	6.20 ± 0.85	6.50 ± 0.91	88.5	NS	0.213
MEP amplitude, mV	4.50 ± 1.22	5.80 ± 1.65	56	0.02	0.502
CMAP amplitude, mV	19.80 ± 4.19	22.30 ± 6.64	91.5	NS	0.187
CMAP latency, ms	3.40 ± 0.37	4.00 ± 0.76	44	NS	0.609
A ratio (MEP/CMAP)	0.24 ± 0.09	0.28 ± 0.11	74	NS	0.342
F-wave latency, ms	27.00 ± 2.07	28.20 ± 2.83	92.5	NS	0.178
F-wave amplitude, mV	0.10 ± 0.04	0.13 ± 0.06	80.5	NS	0.284
CMCT-F, ms	5.20 ± 1.01	4.80 ± 0.90	85.5	NS	0.240

Legend: A ratio = amplitude ratio; BMI = body mass index; CMAP = compound motor action potential; CMCT = central motor conduction time; CMCT-F = central motor conduction time estimated by means of the F-waves; cSP = contralateral silent period; HDRS = 17-item Hamilton Depression Rating Scale; SD = standard deviation; iSP = ipsilateral silent period; MEP = motor evoked potential; MoCA = Montreal Cognitive Assessment; NS = not significant; rMT = resting motor threshold; TMS = transcranial magnetic stimulation; bold numbers = statistically significant *p* values; * Significant after Bonferroni correction.

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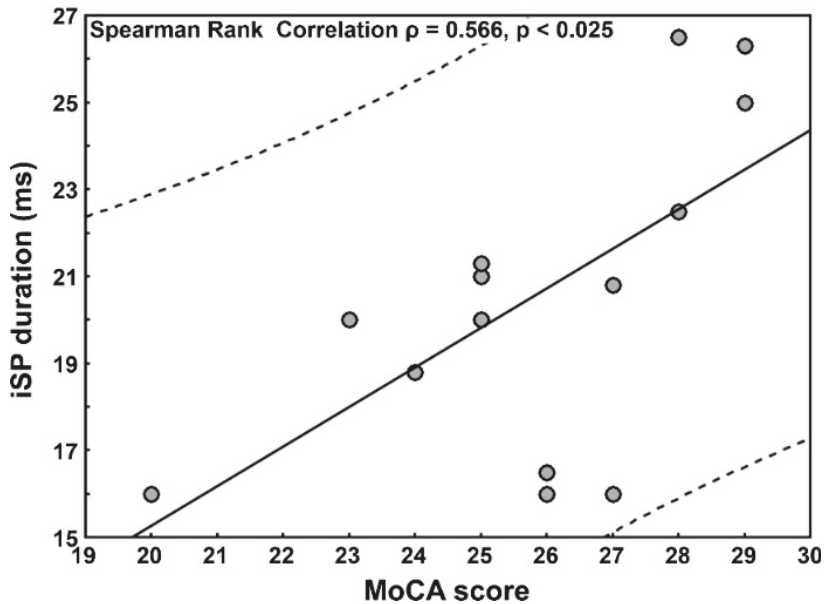
Figure 1. Correlation between MoCA score and iSP duration in patients with celiac disease. Legend: MoCA = Montreal Cognitive Assessment; iSP = ipsilateral silent period; continuous line: linear regression lines; dashed lines: limits within which 95% of observations are expected.

4. Discussion

4.1. Main Findings

In this cross-sectional study, we have first explored non-invasively and in vivo the interhemispheric functioning to TMS in newly diagnosed patients with CD compared to healthy subjects. First, we have confirmed a pattern of intracortical disinhibition in CD, in terms of a shorter cSP duration, thus further supporting the impairment of GABA-mediated intracortical circuits in non-gluten restricted patients [19]. More importantly, we have found an intercortical motor disinhibition, as indexed by a shorter iSP duration, in these patients, suggesting an electrophysiological involvement of the corpus callosum (CC), which positively correlated with worse cognitive performances in asymptomatic patients. Of note, the iSP is thought to be entirely of cortical origin, without a spinal contribution, such as that described for the cSP [30,31,35,40], thus supporting a cortical localization of this disinhibition. Overall, the pathomechanisms underlying these findings seem to be rather complex, also considering the lack of previous investigations and confirmation.

It is worth mentioning that, to date, the integrity of interhemispheric mechanisms of motor cortex excitability and their correlation with cognitive status has been studied in only few neurodegenerative disorders. In mild-to-moderate Alzheimer disease (AD), iSP latency was significantly longer than in controls, whereas iSP duration did not differ between groups. However, no correlation between iSP latency and cognitive function was noted, suggesting that the intercortical motor inhibition might be independent of cognitive impairment in the mild and moderate stages of AD [46]. In another study in AD, the increased iSP latency was not associated with impaired white matter integrity at diffusion tensor imaging, thus hypothesizing that different physiopathological phenomena can account for the reduced transcallosal inhibition observed in these patients [47]. In more severe AD cases, an increased duration of iSP, along with an early onset latency, has been

reported. No correlation was found between cognitive performance and the duration of iSP when the authors mixed both hemispheres, whereas there was a significant negative correlation in the right side if the hemispheres were analyzed separately [48].

In autoimmune and degenerative disorders where the CC is commonly affected, such as MS, the duration of iSP had the highest sensitivity and was not in correlation with MRI-based CC abnormalities in a sample of 49 early patients with relapsing–remitting MS [49]. A subsequent study confirmed that the iSP was altered in early MS and yielded complementary information on subclinical changes. Since pathological brain plasticity has been demonstrated in MS, a compensatory role of the ipsilateral motor and premotor areas was hypothesized [50]. Interestingly, in relapsing–remitting MS patients, the iSP was in correlation with executive cognitive domains, processing speed, visual memory, and physical disability, suggesting that lesioned CC can worsen the level of cognitive impairment and independence status, likely through a "disconnection mechanism" [51].

Finally, in Marchiafava–Bignami syndrome, which is characterized by an early and prominent callosal involvement, a longitudinal clinical, MRI, and TMS study was carried out both in acute stages and six months following the symptoms onset. The baseline assessment demonstrated marked MRI changes, affecting the whole CC. After treatment, symptoms rapidly resolved, along with the neuroradiological changes, except for cognitive impairment. Regarding the iSP, it was not recordable at baseline, whereas it re-appeared at follow-up, also showing a slightly prolonged duration [52].

In the present study, we found an interhemispheric disinhibition, along with a positive correlation between the iSP and MoCA in CD (i.e., a shorter iSP duration with worse MoCA scores). Although the patients were neurologically asymptomatic and their mean MoCA score was still within the normal limits, a statistically significant difference (also after the Bonferroni correction) was found with respect to age- and education-matched healthy subjects. Therefore, these results, although preliminary and in need for further confirmation, might be viewed as an early finding of subclinical cognitive impairment in CD. In this scenario, TMS might identify subclinical changes early and monitor them after the adoption of the GFD.

It is well known that adult subjects with CD may complain some cognitive symptoms, usually in terms of "brain fog", that improve once the GFD is started, although they may re-appear after incidental gluten intake [53,54]. Difficulties in attention and concentration, lapses in episodic memory and word-retrieval, decreased mental acuity, and episodes of disorientation or "confusion" are commonly reported complaints [55]. In some severely affected cases, even an overt dementia can develop [55–58]. Nevertheless, most of the previous studies usually included heterogeneous cohorts, at different disease phases, or without controls. In a very recent pilot study [59], both newly diagnosed CD patients and established CD patients underperformed relatively to controls in visual and verbal memory, whereas the established CD group only underperformed in visual–constructive abilities. These findings confirm that cognitive dysfunction in CD may be already present at diagnosis [59], as observed in our sample. Furthermore, a population-based study found that CD patients had the relevant impairment of reaction time and significantly more anxiety, depression, thoughts of self-harm, and health-related unhappiness [60]. In the same population, advanced neuroimaging showed a significantly enhanced axial diffusivity in several brain areas, including CC [60]. Therefore, it is possible that subclinical neurophysiological changes in interhemispheric transmission might be already evident at the disease onset, as also proposed by our study.

In line with these findings, a recent review of the electrophysiological studies in CD [61], including those using TMS [62], seem to converge on a global pattern of "hyper-excitable celiac brain", that in part improves following a long-lasting GFD. Of note, an overt hyperexcitability is constantly observed in vascular or degenerative dementia [63–65]. Since the GFD may exert some neuroprotection, the diet needs to be adopted as soon as possible, though its effects on CNS manifestations (and in particular cognitive features) are

debated yet [59]. Translationally, the identification of novel and modifiable risk factors is of pivotal relevance for diagnostic, prognostic, and therapeutic purposes.

Psychiatric co-morbidities, and depression in particular, have been frequently associated with CD [66,67]. In our patients, depressive symptoms were significantly higher than in controls, although the mean raw score was suggestive of a mild depression. Furthermore, the difference observed did not resist after the Bonferroni correction. However, depressive disturbances can substantially affect the quality of life and are a reliable marker of poor adherence to the diet [68]. Screening CD subjects for depressive symptoms is therefore crucial, including follow-up visits, to promptly suggest appropriate pharmacotherapy and/or psychological support. Clinically, improvements can be expected only following a long-lasting gluten restriction (>5 years) [69], thus emphasizing the need for sustained and adherent GFD also on neuropsychiatric symptoms of CD.

In this context, TMS has been used to explore inhibitory and excitatory interactions within motor cortical regions in several neuropsychiatric disorders [70]. Specific TMS protocols also provide insights into the regulation of different neurotransmission systems [71]. For instance, rMT and its changes are regarded as an index of membrane excitability of the cortico-spinal neurons and interneurons within M1 [27]. It is increased by drugs blocking voltage-gated sodium channels [72,73], whereas is not affected by drugs acting on GABA [73], glutamate [74,75], or dopamine [76]. TMS also activates inhibitory cortical circuits containing GABAergic interneurons and, among them, the cSP is known to be influenced mainly by GABA [77]. Similarly, transcallosal inhibition represents the spread of an inhibitory signal from a motor cortex to the other [78]. As such, the iSP is a complex phenomenon, being the duration of transcallosal inhibition dependent on a GABA-mediated inhibition. In the present study, the shortening of both the cSP and iSP in patients, along with the correlation between the MoCA and iSP, may provide hints towards the involvement of central GABAergic transmission and a relationship between TMS-measured GABAergic dysfunction and cognitive performance in CD.

From an electrophysiological perspective, both the cSP and iSP durations are also known to be shorter in MDD, a finding in line with earlier studies of abnormal GABA functioning in the frontal lobe of depressed subjects [79]. Although the patients included in our study did not have MDD, the observed changes in mood in the context of an intracortical and intercortical disinhibition might support the involvement of GABA circuitries within the M1 and CC, respectively [80]. However, a correlation between HDRS scores and SPs was not found, and, therefore, additional investigations should be encouraged to also extend the present data in the brain areas more closely associated with the pathophysiology of mood disorders. Recently, rMT was found to be higher in MDD patients compared with healthy controls, while cSP and iSP were significantly shorter in duration [79]. The authors also observed a positive correlation between scores in the Beck Depression Inventory and the rMT, and a negative correlation with cSP duration, suggesting a global hypoexcitability of both pyramidal cortical neurons (increased rMT) and GABAergic control (shortened SPs) within the dominant M1, which is consistent with previous reports of dysfunctional glutamate and GABA in the frontal cortex in MDD [79].

Lastly, the reason why patients exhibited smaller MEP amplitude compared to the controls (although not significant after the Bonferroni correction) remains quite difficult to explain, with a stochastic effect due to the relatively small sample size not excluded. Theoretically, because a peripheral nerve disease can affect patients with CD [81,82], it might be hypothesized that a reduced MEP amplitude could be caused by a peripheral lesion of the motor axons. However, the lack of clinical findings, along with normal motor nerve excitability and conduction, ruled out this possibility. Moreover, the amplitude ratio, as well as rMT, CMCT, MEP latency and duration, were normal, thus confirming the absence of any significant abnormality along the cortico-spinal tract conductivity.

4.2. Limitations

The main limitation, as usually occurs in most studies with TMS, is the relatively small sample size, though the patients were carefully screened and selected, they were homogenous for clinical-serological features and histopathological findings, were all de novo and drug-free, and matched for age and sex with healthy subjects.

Another caveat is that, since TMS provides a functional evaluation of the interhemispheric activities but not of structural changes, a detailed morphological assessment of the cerebral cortex and CC were not performed, thus precluding correlations with neuroimaging data. The same holds true for an extensive neuropsychological battery of tests. Although we have excluded clear neuroradiological abnormalities in all patients, brain CT remains a gross radiological exam, able to detect intracranial calcifications (found in some CD patients) better than MRI, but with quite a low sensitivity and specificity for CC lesions. Therefore, further studies correlating clinical, TMS, and MRI data are needed.

Lastly, although the results have showed some differences in the excitability to TMS between patients and controls, these data should be viewed as only a part of the complex pathophysiological state of the CNS in vivo. Specifically, caution is needed when interpreting these findings as somewhat definitely representative of the status which the TMS variables are able to measure. Therefore, it should be acknowledged that there is ultimately uncertainty over what is precisely being reflected by such differences.

5. Conclusions

An intracortical and interhemispheric motor disinhibition to TMS was observed in de novo, neurologically asymptomatic, CD patients, suggesting the involvement of the GABA-mediated cerebral cortex and transcallosal circuitries. Future studies in larger samples and follow-up during dietary regimen will further support and expand these results in CD and other gluten-related CNS diseases.

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Article

Neurological Evaluation of Patients with Newly Diagnosed Coeliac Disease Presenting to Gastroenterologists: A 7-Year Follow-Up Study

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Abstract: We have previously shown that 67% of patients with newly diagnosed coeliac disease (CD) presenting to gastroenterologists have evidence of neurological dysfunction. This manifested with headache and loss of co-ordination. Furthermore 60% of these patients had abnormal brain imaging. In this follow-up study, we re-examined and re-scanned 30 patients from the original cohort of 100, seven years later. There was significant reduction in the prevalence of headaches (47% to 20%) but an increase in the prevalence of incoordination (27% to 47%). Although those patients with coordination problems at baseline reported improvement on the gluten free diet (GFD), there were 7 patients reporting incoordination not present at baseline. All 7 patients had positive serology for one or more gluten-sensitivity related antibodies at follow-up. In total, 50% of the whole follow-up cohort were positive for one or more gluten-related antibodies. A comparison between the baseline and follow-up brain imaging showed a greater rate of cerebellar grey matter atrophy in the antibody positive group compared to the antibody negative group. Patients with CD who do not adhere to a strict GFD and are serological positive are at risk of developing ataxia, and have a significantly higher rate of cerebellar atrophy when compared to patients with negative serology. This highlights the importance of regular review and close monitoring.

Keywords: coeliac disease; neurological dysfunction; ataxia; headaches; neuropathy; anti-gliadin antibodies; MR imaging; TG6 antibodies

1. Introduction

Coeliac disease (CD) is an autoimmune disorder triggered by the ingestion of gluten in genetically susceptible individuals and it affects 1% of the population [1]. CD belongs to the spectrum of gluten-related disorders that encompass diverse manifestations, including dermatitis herpetiformis (DH) and neurological dysfunction (gluten ataxia, gluten neuropathy, gluten encephalopathy) [2].

To what extent do patients with classic CD presentation suffer from neurological problems is an ongoing enquiry. Research has found evidence of cognitive deficits [3], which may recover by a degree after starting the gluten-free diet (GFD) [4], which CD carries an increased risk of developing vascular dementia [5], and some reports of brain atrophy and white matter lesions alongside neurological problems, which mirror those found in patients presenting neurologically with gluten ataxia/gluten encephalopathy [6,7]. While

this body of research is sometimes complicated by CD cohorts including patients recruited from neurology departments (thus raising questions of generalisability to "typical", gastrointestinal patients), more recently a validation study based on UK national population data bank has replicated some of these, including evidence of cognitive problems and brain white matter changes compared to controls [8].

With almost all of these experiments being single-timepoint, what remains most understudied is the longitudinal outlook of these patients. Importantly, it must be addressed if the GFD is as an effective treatment in stopping these central nervous system injuries from progressing further. Currently, one leading hypothesis for the pathogenesis of these problems focus on similar mechanisms to the gastrointestinal phenotype; which circulating autoantibodies against gluten products exhibit cross-reactivity with key body tissues and cause downstream harm [9]. Gliadin antibodies (AGA) have for example been demonstrated to exhibit reactivity with brain blood vessel structures [10], while AGA status has been correlated with depressive symptoms in healthy controls [11]. The presence of transglutaminase 6 (TG6) antibodies have also been implicated as potentially diagnostic/pathogenic in the development of gluten ataxia [12], with research demonstrating TG6 expression in cerebellar tissue (the primary site of injury in the condition) [13]. Transglutaminase 2 antibodies (TG2), a major diagnostic indicator for CD, have also been found deposited around brain vessel walls in patients with gluten ataxia [14]. These findings raise the question: does achieving negativity for all of these antibodies through the GFD [15] slow or even stop progression of neurological harm in CD patients?

In 2010 we conducted a 3-year prospective study with the primary aim of establishing the prevalence of neurological involvement at the time of diagnosis of CD in patients presenting to gastroenterology clinics with the typical gastroenterological symptoms of CD [16]. We also investigated any association between the presence of circulating TG6 antibodies and neurological deficits. The study demonstrated that out of 100 patients with newly diagnosed CD, 67% had symptoms and/or signs of neurological dysfunction. Sixty percent had abnormal brain imaging, including abnormal MR spectroscopy of the cerebellum in 46% and/or brain white matter lesions over and above what is expected from age in 25%. Forty percent of patients who had circulating TG6 autoantibodies displayed atrophy of subcortical regions on brain imaging, particularly involving the thalamus and the cerebellum, when compared to those patients with no TG6 antibodies.

In this follow-up study we reviewed 30 of the original participating patients 7 years after their baseline assessment. They underwent detailed clinical examination and repeat brain imaging. This investigation aimed to characterise any change in the clinical (neurological) phenotype of these patients, and also to investigate for relationships between antibody status and rate of brain atrophy.

2. Methods

2.1. Patient Selection and Clinical Assessments

The study was based at the Departments of Gastroenterology, Academic Department of Neurosciences and Neuroradiology, Sheffield Teaching Hospitals NHS Trust, Sheffield, UK. The study (research number STH20656) was approved by the local ethics committee and informed consent was obtained from all participants. All 100 patients who had participated in the original study were send an invitation to participate in the follow-up study. The ethics approval allowed for only a single invitation letter to be send to each of the 100 original participants. Thirty patients who responded to this invitation were seen and reassessed and underwent further brain imaging as per the original protocol (see below). Reasons for patients non-responding included: patients had moved away from Sheffield (the original cohort was from the Sheffield catchment area), and the COVID pandemic, which disrupted all non-COVID research in the UK for 9 months. Additionally, the terms of ethical approval did not allow for a second invitation letter to be sent at a later date. All responders were seen.

As per the original protocol, all patients had been diagnosed with CD following gastroscopy and duodenal biopsy [3]. All patients were clinically assessed by a consultant neurologist, including detailed neurological history and examination. The clinical examination included detailed assessment of gait, including ability to tandem walk, stand on each leg in turn and ability to stand with feet together. All patients had serological testing for gluten sensitivity during their attendance (see below).

2.2. Brain Imaging

Patients underwent MR imaging of the brain, using the same scanner (3T Philips Ingenia, AE Eindhoven, The Netherlands) and sequences as in the original study. Acquisitions included volumetric T1-weighted (T1W) and volumetric FLAIR structural imaging, and MR spectroscopy of the cerebellum (vermis). The methodology of the MR spectroscopy acquisition and the cut-offs for abnormal spectroscopic measurements NAA/Cr (N-Acetyl Aspartate to Creatine ratio) have been described previously. The T1W sequence was a 3D "MPRAGE" routine, 0.8 cm^3 isotropic resolution, TR/TE = 10.5/4.8 ms. The FLAIR sequence was a 3D routine, 0.97 × 0.97 × 0.56 cm resolution, TR/TE = 4800/304 ms, TI = 1650 ms.

The original study had indicated that both cerebellar grey matter (GM) and the thalamus may be at risk of atrophy in this patient cohort. Accordingly, volumetry of these regions was performed on the newer scans and re-performed on the baseline scans, to ensure software/workstation comparability. All scans first underwent bias correction using the "N4ITK" package. For estimated of cerebellar GM the "SUIT" pipeline was used (http://www.diedrichsenlab.org/imaging/suit.htm (accessed on 25 March 2021), Figure 1), while thalamic volumes were calculated using FSL's "FIRST" pipeline. Each of these methods were used to produce volumes for the region of interest (ROI). For FIRST this was calculated as the volume of all voxels identified as left/right thalamus in the final, boundary-corrected output. For SUIT this was calculated as the mean*volume of all voxels in the initial cerebellar GM tissue probability map. These "raw" volumes were then calculated as a percentage of one another, per-patient, to reflect what percentage brain volume change (pBVC) had occurred over time. This percentage was normalised with reference to the time which had passed between their two scan dates, to calculate the percentage yearly brain volume change (pYBVC). Finally, FSL's "SIENA" package was also run for an estimation of overall brain volume change over time. This similarly produces a pBVC value, which was also normalised to create pYBVC.

2.3. Serology

Serological testing for IgA transglutaminase 2-TG2 (Phadia, Thermo Fisher Scientific, Uppsala, Sweden), anti-gliadin (AGA) IgG and IgA (Phadia, Thermo Fisher Scientific, Uppsala, Sweden), endomysium-EMA (Werfen, Warrington, UK), and IgG and IgA transglutaminase 6-TG6 (Zedira, Darmstadt, Germany) antibodies was undertaken in all patients during their visit for clinical assessment. All tests were performed at the NHS immunology lab as part of normal clinical care. The exact kits used differed in some instances compared to patient's baseline assessments (both for AGA and TG6 antibodies).
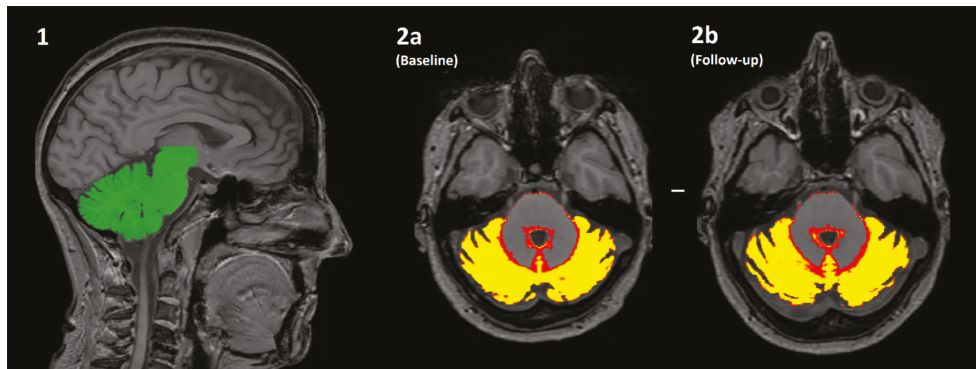
Figure 1. An overview of how the volume of cerebellar grey matter is estimated using the SUIT pipeline. (**1**) The cerebellum is automatically identified (green) and segmented from the original T1-weighted image. (**2a/2b**) The cerebellum is then further segmented into a "tissue probability map", where the value of each pixel is transformed into the % probability that it is brain grey matter. The mean of all pixels with a non-zero value is multiplied by the volume of all such pixels (i.e., the mean*volume of red/yellow regions) for both the baseline (**2a**) and follow-up (**2b**) scans. The difference in values is the estimated change in volume over time, which is further normalised by the actual length of time between scanning sessions to produce percentage yearly brain volume change (pYBVC).

2.4. Statistical Analysis

Baseline neurological symptoms of the returning sample were compared by chi-square analysis to the original whole sample to determine if they were significantly different as a subgroup in some manner. Clinical characteristics were then summarised descriptively in terms of how the rate of neurological symptoms has persisted over time. As the kits used to test for gluten antibodies changed in some instances between timepoints these were not explored individually. Instead, where relevant patients were classified based on if they were either negative for all tests, or positive for any test at follow-up.

For imaging analyses, descriptive statistics were produced to show the rate of brain atrophy over the whole cohort. Statistical analysis then focused on comparing the rate of change (pYBVC) between those patients who still had positivity of any gluten-related antibody at follow-up, compared to those in which all antibodies were within normal levels. Age was first compared between these groups to determine if it was significantly different and therefore should be included as a covariate in subsequent analyses or not. Groupwise testing then compared pYBVC of all cerebellar GM (SUIT), the thalamus (FIRST), and overall brain volume (SIENA) between antibody groups, after visual inspection of histograms was performed to decide if parametric or non-parametric models should be used.

3. Results

3.1. Clinical Characteristics

The baseline and follow-up clinical and serological characteristics of the 30 patients are summarised in Table 1. There were 18 female and 12 male patients. The mean age at baseline (on presentation and diagnosis of CD) was 47.8 (range 20–69) and at follow-up 55 (range 26–76).

Table 1. Clinical and serological characteristics of the 30 patients that participated in the follow-up study both at baseline and at follow-up.

	Baseline (30 Patients)	Follow-Up 7 Years Later (Same 30 Patients)
mean age (range)	47.8 (20–69 years)	55 (26–76 years)
headaches	47%	20%
gait instability	27%	47%
sensory symptoms	10%	10%
gait ataxia on examination	33%	47%
sensory loss	0%	13%
EMA positive	93%	17%
anti-gliadin positive (IgG and/or IgA)	80%	30%
TG6 antibody positive (IgG and/or IgA)	37%	33%
TG2 IgA	100%	20%
one or more of the above serological tests positive	100%	50%
new onset of gait ataxia and positive serology at follow-up	N/A	7/7 100%

EMA, endomysium antibodies; TG6, transglutaminase 6; TG2, transglutaminase 2.

Amongst these 30 patients, at the time of diagnosis of CD (baseline study), clinical history revealed that 14/30 (47%) complained of frequent and intractable headaches, 8/30 (27%) complained of balance problems and 3/30 (10%) of sensory symptoms. At the same time, on examination, 10/30 (33%) had gait ataxia, 3 of which also had nystagmus and one had myoclonic tremor. These figures were not significant (Chi square) to what was observed in the whole cohort of 100 patients from which these 30 patients were re-assessed. Further comparisons of baseline data between the returning cohort and other groupings were therefore not sought.

At follow-up (average 7 years later) the clinical history revealed that only 6/30 (20% from the original prevalence of 47%) complained of ongoing headaches and 4 of these 6 said that their headaches were much better since being on gluten-free diet. Fourteen patients (47%) mentioned gait instability. Seven of these patients had not reported any balance problems at the time of their baseline assessments. Of the remaining 7 patients who had reported balance problems at baseline and at follow-up, 3 said that their balance had improved on GFD, the other 4 stated that it was stable. Sensory symptoms were reported by 3/30 (10%) patients.

On examination at follow-up 17/30 (57% as opposed to 33% at baseline) of patients had mild gait ataxia (tandem walking difficulties). Four patients (13% as opposed to 0% at baseline) had loss of vibration sensation in their feet. Two patients had nystagmus (the same 2 that had nystagmus at baseline) and one had myoclonus (same patient as baseline).

All 7 patients who did not have ataxia at baseline assessment but developed mild gait ataxia at follow-up were positive for one or more serological tests for gluten sensitivity. The remaining 10 patients had ataxia from baseline. Of the 10 patients, 3 were found to have a neuropathy at follow-up neurophysiological assessment, 3 showed improvement of their ataxia on GFD, 3 remained the same and in one the ataxia had completely resolved.

Neurophysiological assessments in the 4 patients with evidence of reduced vibration sensation in their feet, at follow-up, showed a peripheral neuropathy in 3 (2 large fibre and 1 small fibre neuropathy). Therefore, the prevalence of neuropathy in this cohort was 10%.

3.2. Serological Tests

Serological tests at follow-up showed that 15 (50%) of patients were positive for one or more tests signifying gluten sensitivity. These included AGA (9 patients) and/or TG6 antibodies (10 patients). Amongst those patients positive for TG6 and/or AGA antibodies there were 6 (20% of total of 30) who were positive for TG2 antibodies and 5 (17% of total of 30) who were positive for EMA. The mean TG2 titre amongst the 30 patients at baseline was 207 U/mL and at follow-up 6 U/mL (normal <7 U/mL).

3.3. Brain Imaging

Brain imaging was performed in 29 patients; one patient failed to attend their appointment. MR spectroscopy from the vermis (NAA/Cr) significantly improved in 12 patients (40%), remained the same in 10 (33%) and got worse in 7 (23%). Of the 7 patients with worse spectroscopy, 5 had positive serology.

Brain volume analysis was performed on 28 patients, as one subject was excluded due to undergoing a clinical scan routine at their follow-up appointment such that the T1W acquisition was not comparable to their baseline. For the remaining 28 patients, average percentage yearly brain volume change (pYBVC) of the different ROIs was as follows: cerebellar GM = -0.53% (± 0.42); thalamus = -0.49% (± 0.38); whole brain = -0.16% (± 0.15).

Of these 28 patients, 14 still had positivity of at least one gluten-related antibody at follow-up. Those who were still antibody positive were not significantly different in age (mean = 55.3 ± 10.2 years) to those who had become antibody negative (mean = 53.2 ± 11.6 years, independent *t*-test $p = 0.612$). Independent *t*-tests revealed the rate of atrophy of cerebellar GM (i.e., pYBVC) was significantly greater for the antibody-positive group (mean pYBVC = $-0.73\% \pm 0.45$) compared to the antibody-negative group (mean pYBVC = $-0.32\% \pm 0.26$, $p = 0.007$, Figure 2). However, pYBVC was not significantly different between antibody groups for either the thalamus ($p = 0.641$) or the whole brain ($p = 0.486$).
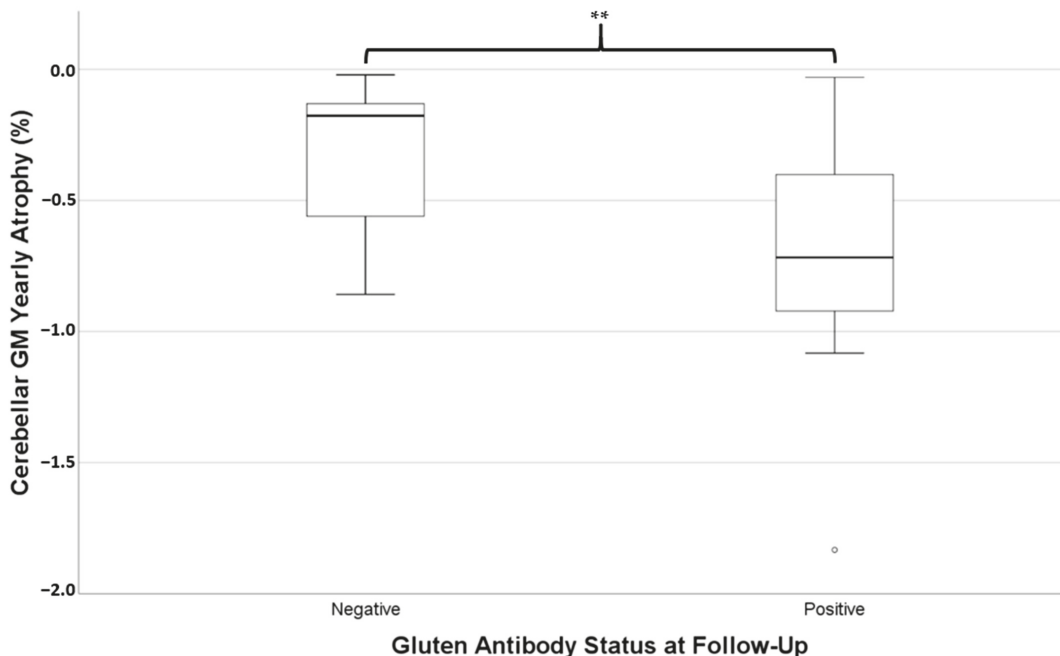
Figure 2. Percentage yearly brain volume change (pYBVC) of cerebellar grey matter, compared between patients who were still positive for gluten-related antibodies at follow-up and those who weren't. A significantly higher rate of atrophy was found in the antibody-positive group (** $p = 0.007$).

4. Discussion

We previously showed that neurological involvement in classic CD is common at the time of diagnosis but is often overlooked [16]. In this follow-up study involving 30 patients from the original cohort, we demonstrate a significant reduction of frequent intractable headaches from 47% to 20% after the introduction of GFD over a period of 7 years. By

contrast, we found a significant increase in the symptoms of gait instability from 27% to 47%. This was also associated with increase in the prevalence of mild gait ataxia on clinical examination from 33% to 47%. In addition, 3 patients (10%) developed a peripheral neuropathy. Of interest is the fact that all of the patients with new onset of mild gait ataxia on examination, had detectable abnormalities in one or more serological tests for gluten sensitivity, suggesting suboptimal adherence to GFD [15]. In fact, overall, 50% of the 30 patients assessed 7 years later still had positive serology for one or more serological markers, although the level was significantly lower than baseline measurements.

We have previously showed that patients presenting with gluten ataxia who adhere to a strict GFD as indicated by the complete elimination of all antibodies, improve [17]. Those who do not go on the diet, worsen and those who go on the diet but are still serologically positive, also worsen, but at a slower pace. Such findings demonstrate that serological positivity may at least be a marker of gluten-sensitive patients who are at continued risk of neurological injury. Indeed, as CD is autoimmune in nature the presence of these antibodies in the blood may be the actual driver of neurological problems, as they react with key tissue sub-types and promote a chronic, harmful inflammatory state [18].

Here, our observations again demonstrate the relevance of serological markers in assessing patients with CD presenting to the gastroenterologists; those patients with CD who developed mild ataxia during this 7-year interval had positive serological markers for gluten sensitivity. As strict adherence to the GFD is thought to lead to elimination of related antibodies [15], this implies inadequate adherence to GFD. The fact that these patients had a very mild gait ataxia as opposed to the severe ataxia we often observe in those patients presenting with gluten ataxia, suggests that a GFD that may not be 100% strict, may nonetheless offer some protection. Patients who present with gluten ataxia to neurologists are also on average 10 years older than those presenting with CD with gastroenterological symptoms [16]. More prolonged exposure to gluten in comparison to patients who are diagnosed with CD 10 years earlier may have a role to play in the development of neurological symptoms.

Patients also underwent repeat brain MRI, and analysis of this provides evidence of the importance of the GFD in preventing progression of neurological outcomes on a physiological level. Here, the rate of brain volume loss was estimated for three regions of interest (ROI); the cerebellar grey matter, the thalamus, and the whole brain. While the thalamus and whole brain analyses did not reveal significant findings, a significantly higher rate of atrophy was found in the cerebellum for those patients who still had antibody positivity at follow-up. The cerebellum was included as a ROI given its known relevance in gluten-related disorders. It is, for example, the primary site of damage in gluten ataxia [2], while the baseline study of this cohort indicated that TG6 antibody positivity was associated with lower cerebellar grey matter volume [16]. The current study shows, for the first time, direct evidence that achieving serological negativity with a strict GFD in patients with CD is associated with an at-least reduced progression of brain atrophy. That this has been shown in a group of patients diagnosed via gastroenterology, further underlines the importance of this observation for all patients with CD.

It is potentially concerning that the two brain ROIs, which were selected because they had been shown to be at risk of atrophy in this cohort—the cerebellum and thalamus—each showed a rate of atrophy almost three times as fast as that of the whole brain. Although those with antibody negativity have some level of protection, a faster rate of atrophy in at-risk ROIs may therefore be persisting even in those on well controlled diets. While it has been shown that different regions of the brain shrink at different rates in heathy ageing [19], this nonetheless presents an important detail for further study as it may compel a need for additional treatment (e.g., immunosuppressive medication).

Fifty percent of the patients assessed in this study had positive serology for one or more serological marker for CD 7 years later. Such an immunological response is associated with slow but definite progression of the ataxia. These observations highlight the importance of regular clinical review with repeat serological testing, not just with EMA

and TG2 antibodies but also with AGA and TG6 antibodies. Of interest is the fact that only 37% of these 30 patients were still under active gastroenterology follow-up.

Two of the patients who participated in this follow-up study were subsequently followed-up in the gluten neurology clinic because of the presence of ataxia and an abnormal brain scan, in combination with positive serology. After a review by the dietitian, a repeat scan and serological testing approximately one year later showed significant improvement on MR spectroscopy associated with clinical improvement. This suggests that there might still be a potential for neurological improvement even in the context of previous imperfect adherence to GFD, assuming that there is still some cerebellar reserve and no permanent atrophy.

Four patients developed symptoms of peripheral neuropathy at follow up and in 3 the neurophysiological assessment showed large fibre neuropathy in 2, and small fibre neuropathy in one. This is in keeping with previous observations that peripheral neuropathy is a late manifestation in patients diagnosed with classic CD [20].

This study has its limitations. Our intention was to try and review as many patients as possible from the original cohort. We only managed to review 30 patients, although we did recruit and assess all patients that contacted us following the invitation letter. The smaller number was a result of the interruption of the study by the COVID-19 pandemic and the fact that many patients had moved out of the Sheffield area. Furthermore, the ethics approval prevented us from attempting to contact the patients for a second time, something that we did consider doing after ease of travelling restrictions due to the pandemic. However, we believe that this cohort of 30 patients is a representative group as indicated by the fact that the neurological symptoms and signs were not significantly different to those observed in the baseline cohort of 100 patients, and age and sex of the cohort at baseline was similar to that of the original group.

Future lines of investigation should seek to replicate these findings with larger cohorts, and where possible, more deeply explore the relationship between neurological symptoms/brain imaging findings and relevant clinical markers, such as specific gluten-related antibodies, other markers of dietary adherence, and also indices of cognition and quality of life. Molecular analysis of regulatory factors and of genes relevant to processes of neuroinflammation, neurodegeneration, and autoimmunity would also be desirable to further identify risk factors and better understand the pathogenesis of these findings.

In conclusion, we showed here that whilst the prevalence of headache improves significantly amongst patients with CD presenting to gastroenterologists, there is evidence of development of mild gait ataxia in some patients who have positive serology for gluten antibodies 7 years later. A significantly higher rate of atrophy was found in the cerebellum of those patients who still had antibody positivity at follow-up. This highlights the importance of regular review and close monitoring using a battery of serological tests. Those patients who remain positive need further dietetic input and closer monitoring.

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

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Article

Frequency and Clinical Aspects of Neurological and Psychiatric Symptoms in Patients with Non-Celiac Wheat Sensitivity

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Abstract: Background: Non-Celiac Wheat Sensitivity (NCWS) is characterized by both intestinal and extra-intestinal symptoms. The study aims to investigate the frequency of neuropsychiatric manifestations in NCWS patients and identify their clinical and demographic characteristics. Methods: 278 clinical records of NCWS patients, diagnosed by a double-blind placebo-controlled wheat challenge between 2006 and 2020, were retrospectively revised. Fifty-two patients with Celiac Disease (CD) and 54 patients with Irritable Bowel Syndrome (IBS) served as controls. Results: 87% of the NCWS patients had an IBS-like clinical presentation. The NCWS group showed a longer duration of symptoms, a higher frequency of positive serum anti-nuclear antibodies than CD and IBS patients, and a higher frequency of DQ2/DQ8 haplotypes and duodenal mucosa lymphocytosis than IBS controls. In addition, 50% of NCWS patients showed neuropsychiatric manifestations, while lower percentages were observed in CD (25%) and IBS (28%) controls. Neuropsychiatric symptoms in NCWS were more frequently associated with the male sex, longer duration of symptoms, and IBS-diarrhea-like clinical presentation. Conclusions: Our data suggest that in patients with IBS-like symptoms and neuropsychiatric manifestations of unknown cause, it could be useful to investigate a correlation of these symptoms with wheat ingestion to identify NCWS patients with this 'atypical' manifestation.

Keywords: non-celiac wheat sensitivity; irritable bowel syndrome; multiple food hypersensitivity; neuropsychiatric symptoms; HLA; duodenal lymphocytosis

1. Introduction

Abdominal pain, bloating, and altered bowel habits motivate up to 25% of all outpatient gastroenterological visits [1]. This clinical presentation is common to Celiac Disease (CD), Non-Celiac Wheat Sensitivity (NCWS), and Irritable Bowel Syndrome (IBS); therefore, differential diagnosis can be challenging.

It is well known that CD symptoms are not limited to the gastrointestinal system but involve the whole body due to the malabsorption determined by the damage to the

small intestine. Similarly, IBS and NCWS can have an extra-intestinal presentation. However, it remains unclear why some patients with CD or NCWS have only gastrointestinal symptoms, while others primarily or exclusively suffer from extraintestinal manifestations.

In particular, neuropsychiatric disorders are often referred to and greatly impact the quality of life of the patients suffering from them. Ataxia and neuropathy are the disorders most frequently reported in untreated CD [2], with several studies and case reports describing an improvement in neurological symptoms after the start of a gluten-free diet (GFD) [3]. By contrast, depression, anxiety, and somatization disorders, together with headache and fatigue, are commonly referred by patients with IBS [4,5].

As concerns NCWS, some data report neurological manifestations similar to CD and equally responsive to GFD [2]. However, most of the studies in the literature do not differentiate between NCWS and CD. Hence, the true prevalence of neuropsychiatric symptoms in each disease is difficult to establish, and few data are available.

Our study aimed to analyze the frequency of neuropsychiatric symptoms in patients diagnosed with NCWS and any possible associations with their demographic characteristics and clinical features.

2. Materials and Methods

The clinical records of patients attending the outpatient centers of three Departments of Internal Medicine (at the "P. Giaccone" University Hospital and the "V. Cervello" Hospital in Palermo, Italy and at the "Giovanni Paolo II" Hospital of Sciacca, Italy) between January 2006 and December 2020, were retrospectively reviewed. These records are currently being uploaded into a computerized database. Among the clinical records already included in the database, there were 424 with a definitive diagnosis of NCWS made by double-blind-placebo-controlled (DBPC) wheat challenge.

Inclusion criteria for NCWS patients were: (a) age >18 years; (b) NCWS diagnosis made by an elimination diet and subsequent DBPC wheat challenge; (c) complete clinical records; (d) follow-up duration longer than 9 months after the initial diagnosis and at least two outpatient visits during the follow-up period. Exclusion criteria of NCWS patients were no compliance with the (a)–(d) inclusion criteria and pregnancy. A total of 278 patients were considered eligible for our study (see Supplementary Figure S1).

Two control groups were recruited in the same centers. The first was composed of 52 CD patients and the second of 54 IBS patients. Both were randomly chosen by a computer-generated method from subjects diagnosed during the same period (2006–2020), and age (±2 years) and sex-matched (±5%) with the NCWS patients.

The following criteria were adopted to diagnose NCWS. Firstly, organic gastrointestinal diseases—in particular CD and WA—were excluded. As a result, all the patients had negative serum anti-tissue transglutaminase (anti-TTG) and anti-endomysial (EMA) IgA and IgG antibodies, absence of villous atrophy in duodenal samples (collected in all subjects carrying the DQ2 or DQ8 HLA haplotypes and in others when clinically appropriate), and IgE-mediated immune-allergy tests negative to wheat (skin prick tests and/or serum specific IgE detection). Inflammatory bowel diseases were also excluded. For details, see Supplementary Materials. After other organic diseases had been excluded, NCWS was diagnosed using the following criteria: the resolution of symptoms after a standard elimination diet excluding wheat, cow's milk, or other foods causing self-reported symptoms, and their reappearance on DBPC wheat challenge, performed as previously described [6,7] (for details, see Supplementary Materials). Other "open" food challenges were also performed.

As regards the control groups, the CD was diagnosed according to the current guidelines [8], and IBS was diagnosed according to the Rome IV criteria [9]. The IBS controls included in this study had been receiving the same elimination diet as the NCWS patients but had not shown any clinical improvement.

As concerns neurological disorders, we only considered self-reported neuropsychiatric symptoms that disappeared on a wheat-free diet and reappeared after wheat ingestion on the DBPC challenge. Our evaluation did not include overt neurological and psychiatric

diseases diagnosed by specific tests (imaging, electroencephalogram, electromyography, electroneurography), or symptoms which could be related to any other risk factors known at the time of our study (i.e., diabetes, vitamin deficiencies, exposure to neurotoxic agents) and not related to wheat ingestion.

For each patient, the following clinical features were analyzed: (1) age at diagnosis, (2) duration of the symptoms (months), (3) body mass index (BMI), (4) presence and kind of IBS-like presentation, (5) presence of dyspepsia, (6) body weight loss (defined as a 10% reduction in body weight in six months or less), (7) anemia (defined as hemoglobin <12 g/dL for women and <13 g/dL for men), (8) presence of concomitant autoimmune diseases, (9) serum anti-nuclear antibody (ANA) positivity (at titer ≥1:80), (10) presence of cow's milk sensitivity, evaluated by DBPC challenge, (11) multiple food hypersensitivity (MFH), other than to wheat and cow's milk, evaluated by open challenges, (12) allergic nickel dermatitis, evaluated by patch tests, (13) concurrent atopy (defined as a history of allergic rhinoconjunctivitis and/or asthma and/or atopic dermatitis), and (14) presence of the HLA DQ2/DQ8 haplotypes. Furthermore, duodenal histology at baseline was evaluated in patients with HLA DQ2/DQ8 or other patients when considered clinically appropriate. Intraepithelial lymphocyte (IEL) counts >25/100 epithelial cells were classified as Marsh 1 lesions [10].

The study was approved by the Ethics Committee of the University of Palermo, Italy. Due to the retrospective design of the study, patients were not consulted. However, we received comments from four patients included in the study during the review and revised our manuscript accordingly. The study was registered at clinicaltrials.gov (registration number NCT04769180).

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) when distribution was Gaussian, and differences were calculated using Student's *t*-test. Otherwise, data were expressed as median and range and analyzed with the Mann-Whitney U test. Fisher's exact test or the Chi-square test were used where appropriate.

3. Results

The demographic and clinical features of the study patients are shown in Table 1.

Table 1. Demographic and clinical features of the patients with NCWS, CD, and IBS.

	NCWS (*n* = 278) (%)	CD (*n* = 52) (%)	IBS (*n* = 54) (%)	*p*
Female Sex	243 (87%)	46 (88%)	47 (88%)	NS
Age at diagnosis (mean ± SD; years)	37.9 ± 12.4	38.9 ± 14.8	40.5 ± 14.3	NS
Duration of the symptoms (median and range; months)	60 (0–612)	12 (1–732)	30 (6–360)	NCWS vs. CD 0.0001 NCWS vs. IBS 0.04
Body Mass Index (mean ± SD)	24.1 ± 5.2	21.6 ± 5.1	26.5 ± 6.2	CD vs. IBS 0.02
Presence of IBS-like symptoms	242 (87%)	38 (73%)	54 (100%)	NCWS vs. CD 0.03 NCWS vs. IBS 0.02 CD vs. IBS 0.0002
Dyspepsia	155 (56%)	38 (73%)	33 (61%)	NS
Body weight loss	70 (25%)	23 (44%)	10 (18%)	NCWS vs. CD 0.01 NCWS vs. IBS 0.01 CD vs. IBS 0.01
Anemia	84 (30%)	31 (59%)	6 (11%)	NCWS vs. CD 0.0001 CD vs. IBS 0.005
Autoimmune diseases	56 (20%)	11 (21%)	7 (13%)	NS
Hashimoto's thyroiditis	42 (15%)	6 (12%)	5 (9%)	NS
Other AD	23 (8%)	5 (10%)	3 (6%)	NS

Table 1. *Cont.*

	NCWS (*n* = 278) (%)	CD (*n* = 52) (%)	IBS (*n* = 54) (%)	*p*
Positive serum ANA	130 (47%)	16 (30%)	3 (5%)	NCWS vs. CD 0.03 NCWS vs. IBS 0.0001 CD vs. IBS 0.0001
Cow's milk sensitivity	177 (64%)	12 (23%)	12 (22%)	NCWS vs. CD 0.0001 NCWS vs. IBS 0.0001
Multiple food hypersensitivity (other than cow's milk)	101 (36%)	2 (4%)	6 (11%)	NCWS vs. CD 0.0001 NCWS vs. IBS 0.0005
Allergic nickel dermatitis	45 (16%)	5 (10%)	4 (7%)	NS
Atopic diseases	93 (33%)	13 (25%)	16 (29%)	NS
HLA DQ2/DQ8 haplotypes	154 (55%)	52 (100%)	16 (30%)	NCWS vs. CD 0.0001 NCWS vs. IBS 0.001 CD vs. IBS 0.0001
Marsh 0	83 (48%)	0 (0%)	7 (100%)	
1	88 (52%)	10 (18%)	0 (0%)	Frequency of Marsh 1 lesion: NCWS vs. IBS 0.02
2	0 (0%)	0 (0%)	0 (0%)	
3	0 (0%)	42 (82%)	0 (0%)	Frequency of Marsh 3 lesion: NCWS vs. IBS 0.0001 CD vs. IBS 0.0001

Abbreviations: NCWS = Non-Celiac Wheat Sensitivity; CD = Celiac Disease; IBS = Irritable Bowel Syndrome; SD = Standard Deviation; AD: Autoimmune Diseases; ANA = Anti-Nuclear Antibodies; HLA = Human Leukocyte Antigen; NS = Non-Significant.

Two hundred and seventy-eight patients were diagnosed with NCWS and 87% were female. In addition, the median duration of symptoms from onset to diagnosis was much longer in the NCWS group (60 months) than in the CD (12 months) and IBS (30 months) groups (NCWS vs. CD *p* = 0.0001; NCWS vs. IBS *p* = 0.04).

Eighty-seven percent of the NCWS study patients showed IBS-like symptoms, a frequency significantly higher than in CD controls (*p* = 0.03). Among the NCWS patients with IBS-like symptoms, 143 (51.4%) had IBS-diarrhea, 36 (12.9%) IBS-constipation, and 63 (22.7%) IBS with alternate bowel movements.

CD patients reported weight loss significantly more often than NCWS and IBS (CD 44% vs. NCWS 25% vs. IBS 18%; *p* = 0.01 for both). Interestingly, NCWS patients showed a significantly higher frequency of weight loss and anemia than IBS controls (*p* = 0.01 and 0.005, respectively). This finding was confirmed by the BMI values (CD 21.6 ± 5.1 vs. NCWS 24.1 ± 5.2 vs. IBS 26.5 ± 6.2; CD vs. IBS *p* = 0.02).

Meanwhile, fifty-six NCWS patients showed one or more concurrent autoimmune diseases. Hashimoto's thyroiditis was the most frequent, being present in 42 NCWS patients. A higher percentage of positive ANA was also found in NCWS patients than in CD and IBS (NCWS 47% vs. CD 30%, *p* = 0.03; NCWS vs. IBS 5%, *p* = 0.0001).

Cow's milk sensitivity was significantly more frequent in the NCWS group (64%) than in CD (23%) and IBS (22%) controls (*p* = 0.0001 for both). Similarly, intolerances towards foods other than wheat and cow's milk were more frequent in NCWS than in the control groups (NCWS 36% vs. CD 4%, *p* = 0.0001; NCWS vs. IBS 11%, *p* = 0.0005).

HLA DQ2/DQ8 haplotypes were significantly more frequent in NCWS than in the IBS controls (55% vs. 30%, *p* = 0.001). Similarly, duodenal mucosa intraepithelial lymphocytosis (Marsh 1 lesion) was more frequent in NCWS than in the IBS controls (*p* = 0.02).

Exactly half of the patients with NCWS reported neuropsychiatric symptoms (139/278), and the frequency of these symptoms in the NCWS group was greater than in the CD and IBS controls (NCWS 50% vs. CD 25%, *p* = 0.002, NCWS vs. IBS 28%, *p* = 0.005). The

demographic and clinical features of the NCWS patients with or without neuropsychiatric symptoms are shown in Table 2.

Table 2. Demographic and clinical features of NCWS patients with neurological symptoms compared to NCWS without neurological symptoms.

	NCWS Patients without Neuropsychiatric Symptoms (*n* = 139) (%)	NCWS Patients with Neuropsychiatric Symptoms (*n* = 139) (%)	*p*
Male sex	10 (7.2%)	25 (18%)	0.02
Age at diagnosis (mean ± SD; years)	37.7 ± 13.1	37.9 ± 11.7	NS
Duration of symptoms (median and range; months)	60 (2–612)	72 (0–564)	0.05
Body Mass Index (mean ± SD)	23.8 ± 4.8	24.3 ± 5.4	NS
Presence of IBS-like symptoms	119 (86%)	123 (89%)	NS
Dyspepsia	84 (60%)	71 (51%)	NS
Body weight loss	28 (20%)	42 (30%)	NS
Anemia	49 (25%)	35 (35%)	NS
Autoimmune diseases	28 (20%)	28 (20%)	NS
Hashimoto's thyroiditis	18 (13%)	24 (17%)	NS
Other AD	13 (9%)	10 (7%)	NS
Positive serum ANA	60 (43%)	70 (50%)	NS
Cow's milk sensitivity	85 (61%)	92 (66%)	NS
Multiple food hypersensitivity (other than cow's milk)	45 (32%)	56 (40%)	NS
Allergic nickel dermatitis	20 (14%)	25 (18%)	NS
Atopic diseases	47 (34%)	51 (37%)	NS
HLA DQ2/DQ8 haplotypes	79 (57%)	75 (54%)	NS
Marsh			
0	42/87 (48%)	41/84(49%)	NS
1	45/87 (52%)	43/84 (51%)	NS

Abbreviations: NCWS = Non-Celiac Wheat Sensitivity; SD = Standard Deviation; IBS = Irritable Bowel Syndrome; AD: Autoimmune Diseases; ANA = Anti-Nuclear Antibodies; HLA = Human Leukocyte Antigen; NS = Non-Significant.

The NCWS patients with neuropsychiatric symptoms were diagnosed later (approximately one year later, *p* = 0.05), and the proportion of men was higher (*p* = 0.02) than in the NCWS group without neuropsychiatric symptoms. Moreover, although the frequency of the IBS-like clinical presentation was similar in the two groups, IBS-diarrhea was observed more frequently among NCWS patients with neurological symptoms (59% vs. 44%, *p* = 0.02). In addition, there was a higher frequency of body weight loss, anemia, and ANA positivity in NCWS patients with neuropsychiatric symptoms than those without, but no statistically significant differences were observed. Finally, neither HLA DQ2/DQ8 positivity nor duodenal inflammation (Marsh 1 lesion) was significantly different in NCWS patients with or without neuropsychiatric symptoms. The frequency of each neuropsychiatric symptom in the three groups is shown in Figure 1.

Headache was the most frequently reported neuropsychiatric symptom in the NCWS group, with a significantly higher percentage than in the two control groups (*p* = 0.005 for both). Fatigue was reported in similar percentages in the NCWS and CD patients, which were higher for both these groups than in IBS controls (NCWS 21.9% vs. CD 19.2% vs. IBS 9.3%, NCWS vs. IBS *p* = 0.03). Myalgia was recorded in 7.9% of NCWS patients with a significantly higher frequency than in the CD and IBS control groups (*p* = 0.03 for both). On the contrary, psychiatric disorders, such as anxiety, depression, and panic attacks, were

more frequent in the IBS group (NCWS 3.2% vs. CD 1.9% vs. IBS 18.5%, NCWS vs. IBS *p* = 0.03, CD vs. IBS *p* = 0.01).
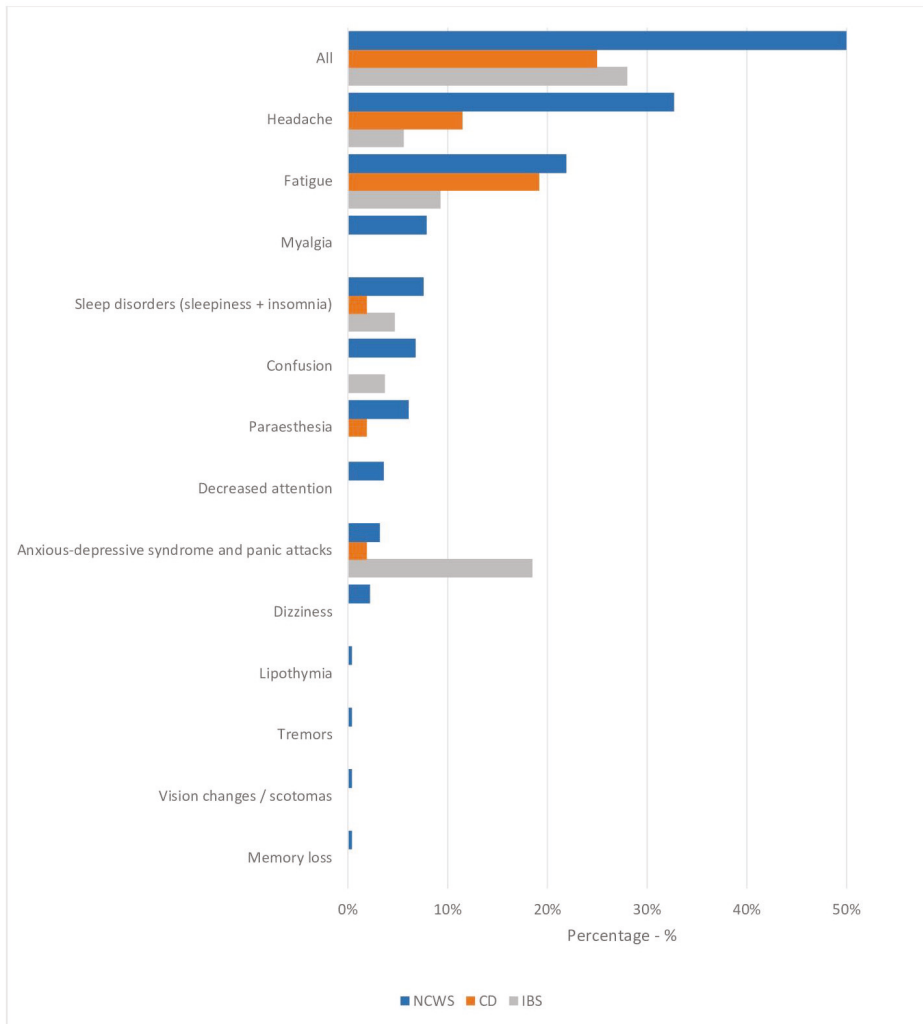

Figure 1. Frequency of each neurologic and psychiatric symptom in NCWS patients (*n* = 278) and in CD (*n* = 52) and IBS (*n* = 54) controls included in the study. Data are expressed as percentages.

4. Discussion

NCWS is an emerging clinical condition that has increasingly attracted the interest of researchers in the last few years. In our study, we retrospectively included 278 patients diagnosed with NCWS by the DBPC wheat challenge. The clinical characteristic of these subjects recalled some of those reported in previous studies. First of all, this condition seems to be much more frequent, or more often self-reported, among women in the fourth decade of life [11–15], and the same trend of prevalence emerged from our study (females 87%, mean age at diagnosis 37.9 ± 12.4 years). Another interesting issue emerging from our study concerns the delay of NCWS diagnosis. The NCWS group showed a statistically significant delay in diagnosis compared to CD (60 vs. 12 months, *p* = 0.0001) and IBS

(60 vs. 30 months, p = 0.04). This delay may be due to the lack of diagnostic markers for NCWS and that the DBPC challenge, which is the current gold standard for the diagnosis, is not easily performed, except in tertiary centers specializing in gluten-related diseases.

As already reported in the literature, at onset, the symptoms of NCWS patients are similar to those of IBS [16]. In our study group, 87% of the patients showed the clinical criteria for an IBS diagnosis. The frequency of autoimmune disease in the NCWS group (20%) was in agreement with the frequency reported in previous studies of our group [7,17], and a higher percentage of ANA positivity was found in the NCWS group than the controls (NCWS 47% vs. CD 30% vs. IBS 5%). MFH frequency was significantly higher in the NCWS patients than in CD and IBS. This is in keeping with our hypothesis that NCWS subjects might be suffering from a non-IgE-mediated food allergy [18]. The NCWS patients had a higher frequency of HLA DQ2/DQ8 haplotypes than the IBS controls (55% vs. 30%) and this percentage is close to that reported in the literature [2,19]. A further point that suggested an inflammatory condition contributing to NCWS pathogenesis was the duodenal mucosa lymphocytosis observed in 52% of the NCWS patients included in the present study, a percentage significantly higher than in the IBS controls.

Due to the prevalent IBS-like clinical presentation in NCWS patients, most previous studies have focused on improving a wheat-free diet on the intestinal symptoms. However, although extra-intestinal symptoms have also been reported in NCWS, few studies have reported their frequency. In addition, no previous studies, to our knowledge, have evaluated the clinical characteristics of NCWS patients with neurological manifestations referred to Internal Medicine or Gastroenterology Units.

We found that 139 (50%) of the NCWS patients showed at least one neuropsychiatric symptom which disappeared on the wheat-free diet and reappeared on the DBPC wheat challenge. Neuropsychiatric symptoms were found to be more frequent in NCWS patients than in CD or IBS patients (respectively NCWS 50% vs. CD 25%, p = 0.002; NCWS vs. IBS 28% p = 0.005). Since the duration of symptoms from onset to diagnosis was longer in the NCWS patients than in CD controls, this might suggest that the duration of wheat exposure could determine a greater risk for developing neuropsychiatric symptoms. However, to the best of our knowledge, there are no other available data about this issue. In fact, in the literature, gluten-related neurological disorders are better described in patients with CD than with NCWS [20]. Nevertheless, another study reported that patients with CD are more likely to develop neurological symptoms earlier than NCWS patients [2].

Among the NCWS patients, there was a higher prevalence of the male sex in the group with neurological symptoms (18% vs. 7.2%, p = 0.02). Of the total 35 male patients with NCWS included in the study, 25 complained of neurological symptoms. This finding agrees with the higher prevalence of male sex observed among the patients analyzed in a recent systematic review, which showed that the majority of gluten-related neurological disorders, proven by histological findings on nervous tissue biopsy or autopsy, affected men [21].

It is also interesting to note that in the NCWS group, the patients with neuropsychiatric symptoms received a later diagnosis (approximately one year later) than those without neurological manifestations. This finding is in keeping with the literature, which reports that CD patients presenting with neurological manifestations are likely to be diagnosed significantly later than those presenting with gastrointestinal symptoms [2].

Very little is known about the pathogenesis of gluten/wheat-related neurological manifestations. Still, several studies have suggested a major role for the emerging concept of a "microbiota-gut-brain axis": according to this hypothesis, there is a close connection between the gut microbiota and the central nervous system, with the former regulating the central nervous system's functioning via neural, immune, and endocrine pathways. Moreover, the gut microbiota might reduce some neurotoxic intermediates or immunogenic wheat proteins and delay the onset or reduce the severity of neurodegenerative disorders [22–24].

One of the etiopathogenetic hypotheses for gluten-related disorders is known as "the leaky gut hypothesis." Gluten is identified as the element that triggers an innate autoim-

mune response, which feeds an inflammatory process affecting the intestinal mucosa, reducing the number of tight junction proteins and increased permeability of the intestinal barrier. This results in the passage of immunogenic molecules into the peripheral circulation. Thus, both the diffusion of gluten-derived peptides in the blood and the immunologic activation driven by other wheat proteins could justify the development of the extra-intestinal manifestations of these diseases, which then take on the characteristics of real syndromes [25–27].

However, in our study, we observed that the NCWS patients with neurological symptoms showed a higher prevalence of IBS-diarrhea symptoms than those without neurological symptoms. This could suggest that symptoms may be due to the malabsorption of trace elements important for proper central nervous system functioning.

As regards the specific symptoms recorded in our study groups, the headache was the most common symptom among NCWS patients (32.7% vs. CD 11.5%, p = 0.02; vs. IBS 5.6%, p = 0.0001), followed by fatigue, which was significantly more frequent in NCWS than in the IBS group (p = 0.03). In addition, myalgia was recorded in 7.9% of the NCWS patients, with a significantly higher frequency than in the CD and IBS control groups. On the contrary, anxiety and depression were more common in IBS than in NCWS patients.

Although our study provides relevant data on the frequency and clinical aspects of the neuropsychiatric manifestations due to wheat ingestion in NCWS patients, its limits must be underlined. First, it is a retrospective study, and the results need to be confirmed in a prospective design. Second, no neuroimaging studies were performed, and we cannot affirm that the neuropsychiatric symptoms were linked to real neuronal damage. Third, we have no data about the patients' intestinal microbiota, changes in which, driven by the wheat-containing or the wheat-free diet, could have important effects on the gut-brain axis. Fourth, our data were not collected in neurology clinics. Almost all our patients had gastrointestinal symptoms as the most relevant clinical presentation. Thus, our estimation of the frequency and severity of the neuropsychiatric manifestations probably applied to patients with mild existing neuropsychiatric symptoms.

The strong point of the study was that it included a large number of NCWS patients diagnosed by DBPC wheat challenge and well defined by additional clinical and laboratory data.

5. Conclusions

In conclusion, we found that 50% of the NCWS patients with mainly gastrointestinal symptoms also suffered from neuropsychiatric manifestations due to wheat ingestion. Male sex, a longer duration of symptoms, and IBS-diarrhea were associated with neuropsychiatric symptoms. Among them, headache, fatigue, and myalgia were the most common. Our data suggest that in patients with IBS-like symptoms and neuropsychiatric manifestations of unknown cause or poorly responsive to drugs, it could be useful to investigate a possible correlation of these symptoms with wheat ingestion to identify patients with CD or NCWS with this 'atypical' manifestation. Further studies will be needed to clarify the pathogenesis of gluten-related neuropsychiatric manifestations and their correlation with the clinical features of NCWS patients to enable an earlier diagnosis.

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