Int. J. Neonatal Screen. **2015**, *1*, 79-88; doi:10.3390/ijns1030079

OPEN ACCESS

International Journal of

Neonatal Screening

ISSN 2409-515X

www.mdpi.com/journal/neonatalscreening

Review

Neonatal Screening for Medium-Chain Acyl-CoA Deficiency—Insights and Unexpected Challenges

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Academic Editor: Ralph Fingerhut

Received: 30 September 2015 / Accepted: 18 November 2015 / Published: 23 November 2015

Abstract: With the implementation of tandem mass spectrometry (MS/MS), neonatal screening for medium-chain acyl-CoA dehydrogenase (MCADD) has been introduced in many screening programs worldwide. Together with phenylketonuria, MCADD is the disorder most frequently diagnosed. Despite undeniable beneficial effects on morbidity and mortality, neonatal screening for MCADD effectively exemplifies the unexpected challenges of increased diagnosis by screening programs. MS/MS-based screening revealed an at least 2-fold higher incidence than expected with a considerable share of individuals showing mild biochemical alterations and/or novel mutations with unknown clinical significance. Whether these individuals are at lower risk to experience metabolic decompensations is a matter of ongoing debate. Defining patients, stratifying them according to their clinical risk, and adopting treatment protocols is an as yet unmet challenge in neonatal screening for MCADD.

Keywords: medium-chain acyl-CoA dehydrogenase; neonatal screening; newborn screening; tandem mass spectrometry; confirmation of positive screening results

1. Introduction

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD; MIM# 201450) is the most frequent defect of mitochondrial β-oxidation, first described in 1976 [1]. MCADD leads to an impaired breakdown of fatty acids and subsequently to a reduced synthesis of ketone bodies during episodes of catabolic stress such as prolonged fasting, intercurrent illness with fever, reduced food intake, or

vomiting. Clinical symptoms may occur at any age, but the majority of patients present during their second year of life. The biochemical hallmark of the disease is hypoketotic hypoglycemia, accompanied by clinical symptoms of lethargy, encephalopathy, seizures, or coma [2].

MCADD leads to a significant morbidity and mortality in undiagnosed patients. Approximately 25% of children with MCADD die during their first metabolic decompensation, and 10% to 30% of the survivors show neurological impairment or developmental delay [3,4]. Moreover, prior to neonatal screening MCADD was an underdiagnosed disorder [3]. Only 12% of patients were diagnosed correctly with MCADD after their first clinical episode. The majority of patients were suspected to have Reye syndrome, idiopathic hypoglycemia, or SIDS [4]. Once diagnosed, follow-up data on symptomatic MCADD patients showed that severe metabolic crisis and death could be prevented by counseling to avoid fasting and by providing emergency procedures (treatment plans) during intercurrent illness [4,5]. In the 1990's, the increasing availability of tandem mass spectrometry (MS/MS)-based technologies facilitated the diagnosis of MCADD and rendered neonatal screening by analysis of acylcarnitines in dried blot spots feasible. Meeting most of the criteria devised by Wilson and Jungner 1968 at the World Health Organisation, neonatal screening for MCADD has been implemented in many newborn screening programs worldwide [6]. Together with phenylketonuria, MCADD is the metabolic disorder most frequently diagnosed in neonatal screening [7,8].

This review aims to summarize insights that have been gained by MCADD neonatal screening and to point out the challenge of defining patients with MCADD.

2. Incidence, Mutational Spectrum and Population Demographics

In clinically diagnosed cohorts, the incidence of MCADD has been observed to be 1:25,000 to 1:43,000 [4,9,10] and to vary considerably among different regions of the world. Based on the carrier frequencies of c.985A>G (p.K329E), the most common mutation of the *ACADM* gene (MIM# 607008), the incidence of MCADD has been estimated to be higher (1 in 13,000) in North-Eastern Europe compared to Southern Europe (1 in 300,000). In individuals of Asian descent, MCADD has been considered extremely rare (1 in 1,061,000) [11]. The mutation c.985A>G accounts for approximately 90% of defective alleles in symptomatic MCADD patients identified before the screening-era: 80% of patients were homozygous for the mutation, further 18% carried the mutation on one allele [12,13].

Identification of MCADD individuals by MS/MS-based neonatal screening revealed an at least 2-fold to 3-fold higher incidence than expected, ranging from 1 in 8500 to 1 in 17,500 [14–19]. In addition, MCADD has been identified in many different ethnicities, some of them showing unexpectedly high frequencies of the disease and distinct mutations, *i.e.*, Japan [20,21], Saudi Arabia [22], Turkey [17,23,24], Hispanics [25]. Notably, the incidence and the mutational spectrum of MCADD vary with the racial and ethnic composition of the population screened [3].

The mutational spectrum of individuals with MCADD identified by MS/MS-based neonatal screening shows a wide variety of mutations. Compared to patients diagnosed after the manifestation of clinical symptoms, in neonatal screening cohorts the prevalent mutation c.985A>G is found less frequently in 40% to 60% of the individuals in a homozygous state accounting for 60% to 80% of defective alleles [14,17,19,24,26]. A further frequent mutation has first been described in neonatal screening cohorts and occurs in approximately 15% of MCADD individuals, c.199C>T (p.Y42H) [14,17,24,27].

In addition, numerous novel mutations have been identified, most of them being missense mutations of unknown clinical relevance [14–17,19,25,27,28].

3. Acylcarnitine Patterns

Octanoylcarnitine (C8), a medium chain length acylcarnitine, is the primary marker for the detection of MCADD. In healthy newborns it is found in very low concentrations (95th percentile 0.14 μmol/L; indicative of MCADD > 0.3 μmol/L) [29]. In addition, secondary markers such as hexanoylcarnitine (C6), decanoylcarnitine (C10), decenoylcarnitine (C10:1), and analyte ratios such as C8/C2 (acetylcarnitine), C8/C6, C8/C10, C8/C12 (dodecanoylcarnitine) are used to increase diagnostic distinction [17,19,24,29]. However, different cut-off policies and diagnostic criteria have been applied by various screening programs and were difficult to compare [6]. Recently, Region 4 Stork (R4S), an international collaboration, generated an extensive database analyzing results of unaffected individuals and confirmed cases in order to delineate comparable tools of pattern recognition on the basis of percentiles rather than analyte cut-off values [30,31].

The age at blood sampling is crucial in detecting MCADD. Thus, sampling is recommended to be performed between 36 and 72 h [10,16,17,32]. In newborns with MCADD, concentrations of C8 show a considerable change with age [16,29,33,34]. Concentrations of C8 have been described to increase during the first 3 days of life and subsequently decline with a highly significant decrease between days 2–3 and 4–5, and days 4–5 and 5–6, respectively [33]. Screening programs that do not take into account this decline of the primary marker analyte might miss individuals with MCADD. In contrast, unaffected newborns show rather constant concentrations of C8 during their first two weeks of life [35].

In addition, several other conditions have been reported to be associated with transient elevations of C8 concentrations and spuriously rise the suspicion of MCADD: low gestational age, low birth weight, pronounced postnatal weight loss, breast feeding, or parenteral nutrition [16,36–39].

4. Correlation between Genotype and Biochemical Phenotype

A correlation between genotype and acylcarnitines has been described for the most frequent *ACADM* genotypes: (a) homozygous for c.985A>G, (b) compound heterozygous for c.985A>G in combination with another mutation except for c.199C>T, (c) compound heterozygous for c.985A>G in combination with c.199C>T.

In general, individuals homozygous for c.985A>G tend to show higher concentrations of C8 and analyte ratios than individuals compound heterozygous for the prevalent mutation c.985A>G. Individuals carrying the mutation c.199C>T on one allele display significantly lower acylcarnitine concentrations. However, considerable overlap between these three groups is found in most studies [16–19]. Among various parameters, analyte ratios C8/C10 and C8/C12 were best to discriminate betweem c.985A>G homozygotes and c.199C>T compound heterozygotes in two studies [24,33].

Even healthy carries of c.985A>G have been described as displaying mildly elevated concentrations of C8 [40,41]. Healthy carriers, however, are not to be burdened by screening programs. In fact, carriers of c.985A>G can be discriminated from compound heterozyotes c.985A>G/c.199C>T with almost negligible overlap by the markers C8 and the ratio C8/C12 [33].

5. Effectiveness of Neonatal Screening

Neonatal screening for MCADD has been shown to be an effective means to reduce the occurrence of severe episodes of decompensation and death compared to unscreened populations [8,42]. The rate of hospital admissions, however, is significantly higher in screened MCADD individuals, with the vast majority of admissions being prophylactic admissions during intercurrent illness [8], and it poses a substantial burden on patients and their families.

Despite early detection of MCADD by neonatal screening, 13 patients have been reported to die during intercurrent illness [14–16,42,43]. Vomiting was described as the common clinical symptom in most cases. Unfortunately, a lack of compliance and a lack of awareness of the disease seemed to contribute to the courses.

Early neonatal death occurs in a small percentage of MCADD patients [44]. These cases might not be prevented by neonatal screening considering age at time of blood sampling, processing, and reporting times, and have been reported on some occasions [10,18,43].

No clinical cases of MCADD are known to have been missed by neonatal screening to date. Recently, two asymptomatic siblings have been described carrying a novel splice site mutation that results in partial missplicing. One of the siblings had shown an unremarkable acylcarnitine analysis at newborn screening [45].

Re-evaluating "borderline" or "false-positive" screening samples by sequence analysis of the entire *ACADM* gene, two studies reported cases that should be designated retrospectively as MCADD cases, and can therefore be regarded as "false negatives" [33,37]. Due to screening policies, however, these individuals could not be contacted to determine their clinical outcome.

By implication, neonatal screening for MCADD produces substantial costs for health care systems. Its cost-effectiveness, however, has been shown to be comparable with well-accepted pediatric health interventions such as routine vaccinations [46].

6. Defining Patients—Do We Know Who Needs Treatment?

Neonatal screening revealed an unexpectedly high incidence of individuals with acylcarnitine patterns indicative of MCADD. Thus, screening programs and health professionals needed to establish diagnostic strategies on who should be regarded as confirmed MCADD cases. For this purpose, biochemical and genetic procedures have been devised. Biochemical tests comprise the detection of characteristic acylcarnitine patterns in confirmatory samples, the excretion of hexanoylglycine in urine, and the demonstration of markedly decreased enzyme activity in leukocytes or cultured fibroblasts. Furthermore, the identification of two "severe" mutations in the *ACADM* gene, *i.e.*, c.985A>G, nonsense mutations, or mutations known from clinically ascertained patients are also widely agreed to be diagnostic [16,19,24,36,47]. These individuals are considered to be at high risk for metabolic decompensation and need to be treated.

However, neonatal screening for MCADD detected numerous individuals with significantly milder biochemical phenotypes and/or novel missense mutations of unknown clinical relevance. It is still a matter of debate whether these individuals can be expected to bear a lower risk of clinical decompensation, or even none at all. To date, all individuals with MCADD detected by neonatal screening are advised to avoid

prolonged fasting and to follow disease management plans in case of intercurrent illness, irrespective of the underlying genotype or biochemical phenotype. Thus, the natural history of presumably "mild" MCADD is unlikely to be unraveled. An attempt to provide assistance in risk assessment has been made by delineating the impact of novel missense mutations on the MCAD protein, either by mapping mutations onto structural models [48–50] or by analyzing the molecular effects of mutations in recombinant variant proteins [49–51]. The underlying molecular mechanism in MCADD has been shown to be misfolding [50,52]. The variant proteins analyzed displayed considerable structural alterations affecting conformation, thermal stability, and catalytic function, yet to various extents [49–51]. Based on these experimental data, it is tempting to classify mutations on the protein level as "severe" or "mild", and to assume that mild mutations might bear a lower risk of decompensation. However, adopting these risk stratifications in clinical practice, *i.e.*, declaring mutations as clinically non-relevant, alleviating emergency procedures, or excluding patients from clinical follow-up, is still challenging.

In MCADD, the risk of decompensation is not primarily determined by genotype but is likely to be associated with various genetic and environmental factors [3]. Notably, all individuals with a positive screening result showed altered biochemical parameters during postnatal catabolic stress indicating a deficient β-oxidation to some extent. It cannot be excluded that these individuals experience sufficient metabolic stress to precipitate metabolic decompensation. This is corroborated by the very recent, first report of clinical symptoms in two newborns with MCADD harboring the presumably "mild" mutation c.199C>T [24].

7. Conclusions

MCADD can be reliably detected by MS/MS-based screening. Screening programs apply heterogeneous policies regarding cut-offs, diagnostic criteria, and confirmatory procedures, though. The incidence of MCADD detected by neonatal screening is unexpectedly high. Mild biochemical alterations, novel genotypes, and ethnic variations have been revealed.

Pre-symptomatic detection by neonatal screening significantly reduces morbidity and mortality in individuals with MCADD by considerably straightforward therapeutic means of reducing overnight fast and providing management plans for intercurrent illness. However, frequent prophylactic hospital admissions during minor illness pose a high disease burden on the patients and their families. The major clinical challenge in future MCADD screening will be to establish a risk stratification to avoid this burden for individuals who might need less treatment, or no treatment at all.

Acknowledgments

The author is indebted to all MCADD patients and their families who took part in the follow-up study of the Bavarian newborn screening program.

Conflicts of Interest

The author declares no conflict of interest.

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