Abnormal Newborn Screening in a Healthy Infant of a Mother with Undiagnosed Medium-Chain Acyl-CoA Dehydrogenase Deficiency

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Abstract A neonate with low blood free carnitine level on newborn tandem mass spectrometry screening was evaluated for possible carnitine transporter defect (CTD). The plasma concentration of free carnitine was marginally reduced, and the concentrations of acylcarnitines (including C6, C8, and C10:1) were normal on confirmatory tests. Organic acids in urine were normal. In addition, none of the frequent Faroese *SLC22A5* mutations (p.N32S, c.825-52G>A) which are common in the Danish population were identified. Evaluation of the mother showed low-normal free carnitine, but highly elevated medium-chain acylcarnitines (C6, C8, and C10:1) consistent with medium-chain acyl-CoA dehydrogenase deficiency (MCADD). The diagnosis was confirmed by the finding of homozygous presence of the c.985A>G mutation in *ACADM*.

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Introduction

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most common inborn error of fatty acid oxidation (Gregersen et al. 2008). MCADD presents with a characteristic acylcarnitine pattern that can easily be identified in dried blood spot samples by tandem mass spectrometry (Oerton et al. 2011), and taken together with the excellent prognosis upon early treatment, MCADD is therefore part of the newborn screening (NBS) program in several countries. In Denmark, MCADD has been part of the NBS program since 2002 (Lund et al. 2012).

Clinical manifestations of MCADD are diverse, but the disease usually presents in the first years of life with hypoketotic hypoglycemia and encephalopathy in relation to an intercurrent illness and/or insufficient energy intake. Undiagnosed/untreated these patients are at high risk of developing a life-threatening metabolic decompensation with sudden death or permanent neurological sequelae (Iafolla et al. 1994; Pollitt and Leonard 1998; Wilson et al. 1999).

Here we present the diagnosis of maternal MCADD on NBS of her newborn child.

Case Report

The proband was born at term after an uneventful pregnancy. Birth weight was 3,816 g. She was the third child of healthy unrelated parents. NBS performed at age 2 days revealed a low blood free carnitine of 6.0 $\mu\text{mol/L}$ (cut off <6.3 $\mu\text{mol/L}$), and the infant was suspected of having carnitine transporter deficiency (CTD). Confirmatory testing was performed according to the Danish

neonatal screening program (Lund et al. 2012) including analysis of plasma free carnitine and acylcarnitines and analysis of urine organic acids. Plasma acylcarnitine profile at age 15 days showed only a slightly decreased concentration of free carnitine (18 $\mu\text{mol/L}$, ref. 24–64 $\mu\text{mol/L}$) excluding CTD. She had a normal acylcarnitine profile (including C6, C8, and C10:1), and the urinary excretion of organic acids was normal, thereby excluding secondary depletion of carnitine. None of the frequent Faroese *SLC22A5* mutations known to segregate in the Danish population (c.95A>G, c.825-52G>A) nor the risk haplotype (Rasmussen et al. 2014) was identified. In parallel with the analyses in the infant, the mother (aged 36 years) was evaluated and turned out to have a normal free carnitine in plasma (30 $\mu\text{mol/L}$, ref. 24–64 $\mu\text{mol/L}$) and highly elevated medium-chain acylcarnitines (C6, C8, and C10:1) consistent with MCADD. Direct sequencing of exon 11 of the *ACADM* gene revealed homozygous presence of the prevalent c.985A>G mutation in the mother, confirming the MCADD diagnosis, whereas the newborn child was found to be heterozygous for the c.985A>G mutation.

The mother denied having any symptoms related to MCADD, and specifically she reported a normal fasting tolerance. However, she had never had any severe infections or diseases, and when she was a child, her parents used to give her high-carbohydrate drinks during intercurrent illness, which may have prevented metabolic crises.

Her diet was evaluated by a metabolic dietician, and it turned out that her intake of fat was 35.8%, and she was recommended to reduce it to a maximum of 30%. Furthermore, she was instructed in following a specific high-carbohydrate regimen in case of illness.

The mother had six healthy siblings who all were offered biochemical evaluation. None of the four sisters that turned up for evaluation had acylcarnitine profiles consistent with MCADD. Two sisters, however, had slightly elevated concentrations of C8 acylcarnitine, one of whom was later found to be heterozygous for the c.985A>G mutation in *ACADM*. Molecular testing was not performed in the remaining three sisters.

Discussion and Conclusions

We report a case of an asymptomatic MCADD patient homozygous for the c.985A>G mutation detected incidentally through NBS due to low free carnitine. This result on NBS in the newborn most likely reflects poor carnitine stores in the mother. However, in our case the mother presented with low-normal free carnitine at the initial evaluation 16 days after delivery. Though the relation between free carnitine in plasma and carnitine stores is unclear, this result does not support low carnitine stores in

the mother. It is well described that plasma carnitine decreases during pregnancy from the 12th week of gestation to term and may reach concentrations half the normal in healthy nonpregnant women. The decrease in total carnitine is mainly caused by a decrease in free carnitine (Schoderbeck et al. 1995) and is thought to be the consequence of a reduced rate of carnitine biosynthesis, possibly because of an inadequate iron status (Keller et al. 2009) or because of a low availability of precursors for carnitine (Ringseis et al. 2010). Interestingly, one study reported a complete normalization of plasma carnitine one month after delivery (Marzo et al. 1994), and our finding of low-normal free carnitine 16 days after delivery may reflect a partial normalization to concentrations often seen in patients with MCADD.

The finding of abnormal concentrations of specific acylcarnitines and low free carnitine on NBS has revealed various types of inborn errors of metabolism in undiagnosed mothers. Thus, maternal MCADD (Leydiker et al. 2011), CTD (De Biase et al. 2012; El-Hattab et al. 2010; Lee et al. 2010; Lund et al. 2012; Schimmenti et al. 2007; Vijay et al. 2006), glutaric acidemia type I (Crombez et al. 2008), and combined homocystinuria and methylmalonic aciduria (Lin et al. 2009) have all been detected through NBS by the finding of decreased free carnitine in the newborn. In addition, elevated specific acylcarnitines in newborns have revealed maternal 3-methylcrotonyl-CoA carboxylase deficiency (Gibson et al. 1998; Koeberl et al. 2003; Lund et al. 2012), very long-chain acyl-CoA dehydrogenase deficiency (McGoey and Marble 2011), holocarboxylase synthetase deficiency (Nyhan et al. 2009), and multiple acyl-CoA dehydrogenation deficiency due to a riboflavin transporter gene defect (Chiong et al. 2007; Ho et al. 2011). These cases illustrate the importance of taking a detailed maternal history and performing biochemical evaluation with acylcarnitine profile and urine organic acids and when appropriate molecular genetic follow-up in mothers of newborns with abnormal screening results if confirmatory testing shows that the newborn is normal.

After the introduction of MCADD to the NBS program, it has become clear that the incidence of MCADD is much higher than previously thought (Andresen et al. 2001; Maier et al. 2009; Maier et al. 2005). The incidence of MCADD detected by NBS in Denmark is 1 in 8,954, whereas the incidence of clinically presenting MCADD in Denmark during the preceding 10 years before screening was only 1 in 39,691 (Andresen et al. 2012). One explanation for this discrepancy is that the genotypes of the screened population differ from the genotypes in the clinically detected population. Thus, genotypes that have not been identified in clinically presenting cases have been identified in a significant number of screened infants, and

these genotypes have been associated with a milder biochemical phenotype (Andresen et al. 2012). Importantly, it has been shown that the proportion of newborns with the prevalent c.985A>G homozygous genotype is approximately 50% in a screened population (Andresen et al. 2012) as compared with 80% in a clinically presenting population (Tanaka et al. 1992), supporting the notion that c.985A>G homozygous MCADD has a higher penetrance than most other MCADD genotypes. This is also reflected in the biochemical phenotype, where c.985A>G homozygous newborns have higher mean and median C8 carnitine levels than newborns with other *ACADM* genotypes (Andresen et al. 2012; Oerton et al. 2011).

The correlation between genotype and clinical phenotype in MCADD is not clear (Andresen et al. 1997; Arnold et al. 2010). Several asymptomatic patients being homozygous for the c.985A>G mutation have been reported (Andresen et al. 2012; Duran et al. 1986; Leydiker et al. 2011). Identification of the asymptomatic mother in the present study represents one further example that c.985A>G homozygous individuals can remain without recognized symptoms until adulthood and that this can also explain some of the discrepancy between the number of clinically diagnosed patients with MCADD and the number of newborns identified by screening (Andresen et al. 2012).

On the other hand others have reported MCADD presenting in adulthood, some with fatal outcome (for review see Lang 2009) stressing the importance of diagnosing these individuals. It may also be of note that both our case and a similar case (Leydiker et al. 2011) are homozygous for the c.985A>G, indicating that low free carnitine in newborns requires this classic genotype in the mother.

In conclusion, the detection of asymptomatic MCADD and other maternal metabolic disorders is important in order to initiate proper management, family screening, and prevention of complications as sudden deterioration can occur with these disorders at any age. Our case underlines the importance of thorough biochemical evaluation of mothers of newborns with reduced free carnitine on NBS if confirmatory testing shows that the newborn is normal.

Synopsis

Thorough biochemical evaluation of mothers of newborns with a reduced free carnitine on NBS is important if confirmatory testing shows that the newborn is normal.

Conflict of Interest

Lise Aksglaede, Mette Christensen, Jess H Olesen, Morten Duno, Rikke KJ Olsen, Brage S Andresen, David M

Hougaard, and Allan M Lund declare that they have no conflict of interest.

Compliance with Ethics Guidelines

This article does not contain any studies with human or animal subjects performed by any of the authors.

Author Contribution

LA: responsible for collecting medical records, gathering relevant literature, and drafting the article

MC, JHO, and DMH: performing biochemical analyses and revising the article critically for important intellectual content

MD, RKJO, and BSA: performing genetic investigations and revising the article critically for important intellectual content

AML: clinical examination and follow-up of case and revising the article critically for important intellectual content

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