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Case Report

MCAD-Deficiency with Severe Neonatal Onset, Fatal Outcome and Normal Acylcarnitine Profile

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Abstract: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is an autosomal recessively inherited disorder of fatty acid oxidation with a potentially fatal outcome in undiagnosed patients. The introduction of tandem mass spectrometry into newborn screening (NBS) has led to the inclusion of MCADD in NBS in many countries, which has resulted in a significant reduction of morbidity and mortality. We report a child with MCADD presenting neonatally with apnoea and heart arrest. Despite intensive efforts to rescue the child, including reanimation for 90 min, the child died at the second day of life. Autopsy revealed fatty liver and also fat storage in heart muscle, which was suggestive of a fatty acid oxidation defect. However, acylcarnitines determined from stored EDTA blood were not suggestive of MCADD. Nevertheless, a subsequent whole exome sequencing analysis revealed homozygosity for the ACADM gene c.1084A>G/p.Lys362Glu mutation.

Keywords: medium-chain acyl-CoA dehydrogenase deficiency; MCADD; newborn screening; NBS; dried blood spots; DBS

1. Introduction

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most frequent disorder of fatty acid β-oxidation. It is an autosomal recessively inherited disorder, with a potentially fatal outcome in undiagnosed patients. The introduction of tandem mass spectrometry into newborn screening (NBS) has led to the inclusion of MCADD in NBS in many countries. The introduction of MCADD screening resulted in an at least two-fold increase of the incidence of the disorder, compared to the pre-screening era (for review see Reference [1]). In addition, NBS for MCADD has resulted in a significant reduction of morbidity and mortality. Before the introduction of NBS, 84% of clinically suspected cases showed somnolence and coma. Mortality was approximately 20%, and 40% retained neurological symptoms after the first metabolic crisis [2]. Protein misfolding leading to thermal lability of the variant MCAD protein has been described as a frequent reason for reduced MCAD activity, and hence is probably the most frequent underlying cause of MCADD [3,4]. The differences in genotype distribution of the pre- and post-screening era have been described by several groups [5–7], as well as the problem

of definite confirmation of the presumptive diagnosis [8], or the prediction of the risk of metabolic decompensation in early diagnosed patients [9,10]. In addition to the increased number of cases detected by newborn screening, there are also a few cases that were missed by newborn screening [11], or that had a fatal outcome even before the newborn screening result had become available [12]. Neonatal onset of symptoms with an often fatal outcome has been previously described [13–15]. Here, we describe a case with a fatal outcome and a normal acylcarnitine profile in an EDTA-whole blood sample, which was spotted onto filter paper after storage at 4 °C for 10 weeks.

2. Case Report

We report a child with MCADD presenting neonatally with sudden apnoea at the second day of life, followed by the deterioration of circulation and heart arrest. Despite intensive efforts to rescue the child, including reanimation for 90 min, the child died at the second day of life. Until the time of apnoea, the child was unremarkable. Oxygen saturation after birth was recorded to be 97%, APGAR 9/10/10. There was no risk of infection. A dried blood sample for NBS was not taken at that time. The only left-over specimen of the patient was EDTA blood, taken at the first day of life and stored for 10 weeks at 4 °C. Autopsy revealed discreet intracytoplasmatic vacuoles within the heart and prominent vacuoles in the liver, which could be confirmed as fat droplets by Sudan staining and electron microscopy. The fat was stored in between organelles, inside the endoplasmic reticulum, and inside the mitochondria. The number of mitochondria was normal and cristae showed no crude alterations. Staining with Sudan also showed vacuoles in the heart muscle, which resembled fat storage as well. These results were clearly suggestive of a fatty acid oxidation defect. However, acylcarnitines determined from this specimen were totally normal and not especially suggestive for MCADD deficiency. Nevertheless, a subsequent whole exome sequencing analysis revealed homozygosity for the ACADM gene c.1084A>G/p.Lys362Glu mutation.

3. Methods

Acylcarnitines were measured within the routine NBS, in multiple reaction monitoring (MRM) mode on a Waters XEVO-TQD, using the neobase test-kit from Perkin Elmer (Turku, Finland). Whole exome sequencing was performed using the Agilent SureSelectXT Kit with paired-end sequencing on an Ilumina HiSeq2500 System (Zurich, Switzerland) with "paired-end sequencing".

4. Results

Acylcarnitines measured from stored EDTA blood were normal, and not especially suggestive for MCADD (see Table 2 and Figure 1). Autopsy revealed fatty liver (Figure 2) and also fat storage in the heart muscle, which was suggestive of a fatty acid oxidation defect. In order to estimate the effect of prolonged storage of EDTA blood at 4 °C before spotting it onto filter paper, a blood sample of a healthy volunteer was also stored for 10 weeks. One aliquot of this sample was spotted directly onto filter paper, and a second aliquot was spotted after storage. Results are presented as % of direct measurement in Table 2.

Table 1. Concentration of acylcarnitines from stored EDTA blood of the patient. (Concentrations in µmol/L).

Compound	Patient *	Reference Range #	Median #	% of Direct Measurement
Free carnitine	25	5–60	15	145
Acetylcarnitine (C2)	3.2	6–60	18	55
Propionylcarnitine (C3)	0.24	<6.0	1.5	40
Butyrylcarnitine (C4)	0.07	<1.0	0.25	63
Isovalerylcarnitine (C5)	0.04	<0.5	0.11	94
Hexanoylcarnitine (C6)	0.04	<0.2	0.04	13
Octanoylcarnitine (C8)	0.16	<0.6	0.04	29
Decanoylcarnitine (C10)	0.00	<0.3	0.05	33
Decenoylcarnitine (C10:1)	0.01	<0.2	0.03	80

Table 2. *Cont.*

Compound	Patient *	Reference Range #	Median #	% of Direct Measurement
Dodecanoylcarnitine (C12)	0.01	<0.3	0.07	95
Tetradecanoylcarnitine (C14)	0.01	<0.5	0.18	49
Tetradecenoylcarnitine (C14:1)	0.01	<0.3	0.07	67
Tetradecadienoylnoylcarnitine (C14:2)	0.01	<0.05	0.01	67
Hexadecanoylcarnitine (C16)	0.25	<8	2.9	69
Hexadecenoylcarnitine (C16:1)	0.01	<0.7	0.22	56
Octadecanoylcarnitine (C18)	0.2	<2.2	0.82	81
Octadecenoylcarnitine (C18:1)	0.06	<4.0	1.5	66
Octadecadienoylcarnitine (C18:2)	0.02	<0.9	0.15	63

* mean of duplicate determination; # for healthy newborns 2–6 days old.

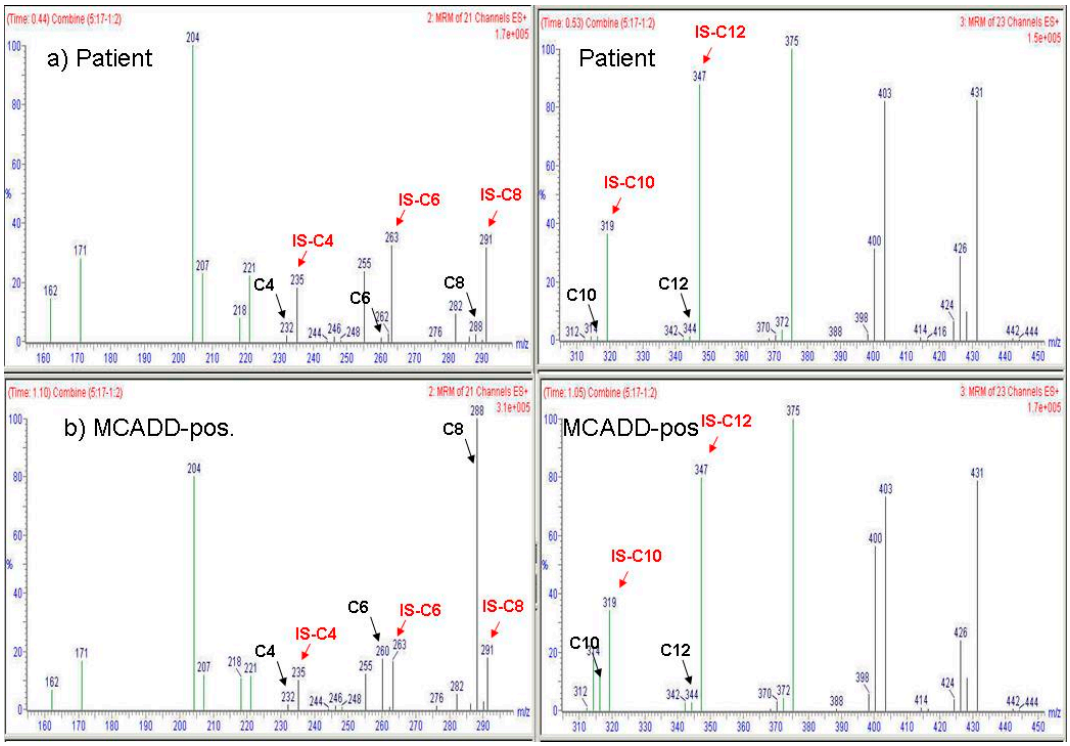

Figure 1. (**a**) Acylcarnitine profile of the patient (Fn); (**b**) Acylcarnitine profile of a typical MCADD-positive newborn screening result.

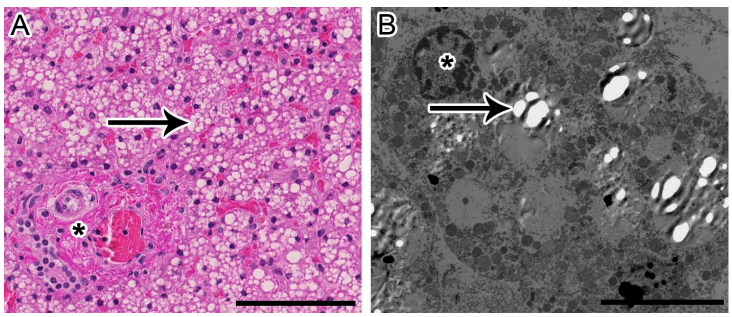

Figure 2. (**A**) H & E staining of the liver, showing unremarkable portal fields (asterisk) but extensive, fine, and coarse intracytoplasmic droplets (arrow; scale bar 100 μm); Electron microscopy (**B**) identified these droplets (arrow) as predominantly intracytoplasmic lipid droplets (nucleus of a cell shown by asterisk; scale bar 10 μm).

5. Discussion

Although newborn screening normally detects patients with MCAD deficiency pre-symptomatically, there are a few cases reported with neonatal onset already at the first days of life, before the newborn screening results are available. In most cases, the acylcarnitine profiles are typical for MCADD, with elevated medium chain acylcarnitines, especially octanoylcarnitine. The case we present here also showed an early neonatal onset with a fatal outcome; however, the acylcarnitine profile was totally normal. Even if we corrected the results for the percentage of degradation, which were calculated from the two aliquots of the same EDTA blood, spotted onto filter paper directly and after 10 weeks of storage, medium-chain acylcarnitines would have still been normal, or slightly elevated at the most. Either way, this case was not in accordance with the clinical severity. In addition, we could not see any unspecific elevations normally found in samples that have been taken under suboptimal conditions, e.g., post mortem.

6. Conclusions

This case report illustrates that (a) a normal acylcarnitine profile cannot exclude MCADD; and (b) that NBS for MCADD cannot always prevent a fatal neonatal outcome.

However, the most astonishing thing about this case is the coincidence of both.

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

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