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RESEARCH REPORT

Lethal Undiagnosed Very Long-Chain Acyl-CoA Dehydrogenase Deficiency with Mild C14-Acylcarnitine Abnormalities on Newborn Screening

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Abstract Newborn screening identifies patients with very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency with disease-specific acylcarnitine profiles. We here present a patient who died at 16 months during a gastrointestinal infection because of undiagnosed VLCADD. The primary acylcarnitine profile on newborn screening performed at 55 h of life revealed C14-acylcarnitine values and ratios within the 1st percentile VLCAD disease range and C12-acylcarnitine values and ratios within the 10th percentile disease range. The acylcarnitine cumulative percentiles in neonatal dried blood spots analyzed by tandem mass spectrometry have been obtained by participants of the Region 4 Stork collaborative project. A secondary screen was requested by the screening laboratory as a result of the initial screen and was normal on day 8 of life. With the initial acylcarnitines only within the 1st–10th percentile disease range, newborn screening for VLCAD deficiency was in the end considered normal. The most important lesson learned is that acylcarnitine profiles from healthy newborns during catabolism and VLCAD-deficient patients can in certain cases not be distinguished by any means. With a known high incidence of false positive cases for VLCADD on newborn screening, it finally remains unknown, whether forced anabolism in the first days of life may result in normal acylcarnitine profiles in VLCAD-deficient patients resulting in missed cases and false negatives on newborn screening. Our observations are of great significance since they demonstrate the limitations of acylcarnitine analysis as screening tool for VLCAD-deficiency.

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Introduction

Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is one of the fatty acid oxidation defects identified by expanded newborn screening programs. Acylcarnitine analysis reveals increased C14-acylcarnitine values and ratios with C14:1-acylcarnitine as the disease-specific marker. Only recently it has been reported that healthy newborns also can present with mildly elevated C14-acylcarnitines and identical profiles during significant catabolism in the first three days of life (Spiekerkoetter et al. 2010). Based on further enzymatic and molecular workup, a false-positive ratio of 1:2,600 has been postulated for VLCADD on newborn screening when taking also mild C14-acylcarnitine elevations into account (Spiekerkoetter et al. 2010). Therefore, functional or molecular confirmatory diagnosis is considered essential to correctly identify affected patients (German AWMF Guidelines 2010). In contrast to these publications on false positive cases, little is known on the frequency of false negative and missed VLCAD-deficient cases (Ficicioglu et al. 2010).

VLCADD poses a risk of death in early infancy, but the condition is generally treatable, with a good outcome after timely diagnosis (Wilcken 2010). Implementation of newborn screening has led to an increasing incidence of VLCADD (Arnold et al. 2009) and a great number of asymptomatic children among those identified. Therefore, it has been proposed that milder phenotypes may have been

previously missed by clinical diagnosis. There are also isolated reports on asymptomatic adult patients identified with fatty acid oxidation defects by family screening (Spiekerkoetter et al. 2003). Only recently, an affected asymptomatic mother with VLCADD was identified through newborn screening in her baby (McGoey and Marble 2011). Currently it is widely debated whether mild phenotypes only present with mild acylcarnitine elevations and can be distinguished from more severe phenotypes by biochemical markers. Other important questions are whether these predicted asymptomatic/mild phenotypes are at all at risk of severe metabolic derangement during catabolism and how patients at risk can be safely identified.

Case Report

The boy was born after an uneventful pregnancy at 40 weeks of gestation. Birth weight was 3,330 g. At 55 h of life newborn screening was performed demonstrating mildly elevated C14- and C12-acylcarnitines as presented in Table 1. Secondary acylcarnitine screening, as requested by the screening laboratory, was performed on day 8 and revealed a normal acylcarnitine profile. Based on the mild elevations on primary screening with C14- and C12-acylcarnitine concentrations within the 1st percentile and 10th percentile VLCAD disease range, VLCADD was excluded.

At 16 months of age, the boy developed a gastrointestinal infection with fever, vomiting and diarrhea. After 24 h of nearly no food intake, the parents found him lethargic presenting with rhythmic convulsions of arms and legs. At hospital admission, status epilepticus, dehydration and hypoglycemia with a blood glucose of 0.94 mmol/l were diagnosed. In addition, metabolic acidosis, elevated lactate, creatine kinase, CKMB, and transaminases were documented. Coagulation tests demonstrated disseminated intravascular coagulation. Organic acid analysis in urine collected during this episode presented mild ketonuria with significantly increased excretion of unsaturated and saturated dicarbonic acids. Acylcarnitine analysis was performed approx. 20 h after start of infusion therapy and documented mildly increased C14:1-acylcarnitine of 0.27 μmol/l (<0.2), C14:2-acylcarnitine of 0.11 μmol/l (<0.1) with a normal free carnitine of 31.2 μmol/l (15–60 μmol/l). On admission, cerebral MRI demonstrated severe cerebral edema. Cardiomyopathy developed within the next 2–3 days. On day 10 after hospital admission, the patient was diagnosed with an EEG zero line and died.

Because of the metabolic markers, diagnostic work up with respect to a fatty acid oxidation disorder was initiated (Liebig et al. 2006). Palmitoyl-CoA oxidation in lymphocytes revealed an activity of 0.50 ± 0.27 nmol/mg/min in three different runs (normal controls 7.76 ± 2.16, $n = 110$). Residual enzyme activity of 6% clearly suggests VLCADD. Sequencing of the complete VLCAD gene delineated compound heterozygosity for the mutations

Table 1 Acylcarnitine values of the index case, normal reference values, and disease ranges according to the literature (McHugh et al. 2011), cut-off levels of the screening laboratory (in μmol/l)

Analyte	Patient	Normal (McHugh et al. 2011)	Disease range (McHugh et al. 2011)				Normal (screening laboratory)
		99 percentile	1 percentile	5 percentile	10 percentile	50 percentile	99 percentile
C16	4.46	6.0	n.s.	n.s.	n.s.	n.s.	7.0
C2	31.40	52	n.s.	n.s.	n.s.	n.s.	76
C14:1	**0.45**	0.37	0.41	0.71	0.83	1.8	0.36
C14:1/C16	0.10	n.a.	0.059	0.18	0.22	0.41	n.a.
C14:1/C2	0.01	n.a.	0.016	0.025	0.030	0.089	n.a.
C14	0.32	0.5	0.24	0.50	0.62	1.3	0.40
C14:2	0.07	0.09	0.042	0.079	0.10	0.24	0.10
C12	0.47	0.41	0.096	0.26	0.34	0.62	0.47
C12:1	0.14	0.27	0.035	0.083	0.13	0.34	0.29
C14:1/C12:1	3.21	n.a.	1.1	1.6	2.1	5.2	5.0
C16-OH	0.04	0.08	n.s.	n.s.	n.s.	n.s.	0.07

n.s. – no specific disease ranges for VLCADD available in McHugh et al. (2011)

n.a. – not available in McHugh et al. (2011) or from the screening laboratory

Overlap between patient's values and disease range values are marked in gray

Abnormal patient values according to the cut-off values of the screening laboratory are marked in bold

829_831delGAG (237Edel) and 1370T>A (I417N). The mother was heterozygous for the mutation 1370T>A, the father was heterozygous for the mutation 237Edel. The deletion is expected to have significant effect on protein structure and function and has to be considered severe. The missense mutation has not previously been reported and thus, its clinical relevance is unknown.

It is an important task to generate an objective definition of cut-off target ranges to be applied to newborn screening. Acylcarnitine cumulative percentiles in neonatal dried blood spots analyzed by tandem mass spectrometry have been obtained by participants of the Region 4 Stork collaborative project (as of December 1, 2010) (McHugh et al. 2011). According to these data, the initial C14-acylcarnitines and ratios in our patient are within the 1st percentile VLCAD disease range, whereas C12-acylcarnitines and ratios are within the 10th percentile VLCAD disease range (Table 1). An important lesson learned is that acylcarnitine profiles from healthy and VLCAD-deficient patients in some cases cannot be clearly distinguished. Whereas, previous reports documented that a normal second screen does not exclude VLCADD or other long-chain fatty acid oxidation defects, if the first screen was conspicuous (Boneh et al. 2006; Schymik et al. 2006), this case report in addition points out, that also the primary screen may be difficult to interpret. With a known high incidence of false positive cases for VLCADD on newborn screening, it finally remains unknown, whether forced anabolism in the first days of life may also result in normal acylcarnitine profiles on newborn screening resulting in missed cases and false negatives.

In conclusion, the case presented here is of great relevance in two respects. First, acylcarnitine profiles from healthy newborns and VLCAD-deficient patients can in certain cases not be distinguished demonstrating the limitations of acylcarnitine analysis as screening tool for VLCAD-deficiency. Second, mild elevations do not necessarily suggest mild VLCADD and do not exclude life-threatening metabolic derangement during severe catabolism.

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Take-Home Message

Acylcarnitine profiles from healthy newborns during catabolism and VLCAD-deficient patients can in certain cases not be distinguished, demonstrating the limitations of acylcarnitine analysis as the screening tool for VLCAD-deficiency.

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