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CASE REPORT

Missed Newborn Screening Case of Carnitine Palmitoyltransferase-II Deficiency

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Abstract Carnitine palmitoyltransferase-II (CPT-II) deficiency can be detected through newborn screening with tandem mass spectrometry. We report a 4-year-old patient with rhabdomyolysis due to CPT-II deficiency, which was initially missed by newborn screening. The patient presented with a 2-day history of fevers, upper respiratory infection, diffuse myalgia, and tea-colored urine. Her medical history was notable for frequent diffuse myalgia when ill. She was demonstrated to have homozygous mutation c.338C>T, p. S113L in *CPT2*, which is typically found in the adult-onset, myopathic form of the disease. An unknown number of CPT-II deficient patients with normal newborn screening have not yet presented to medical care with the adult-onset, myopathic form of disease. We conclude that (1) not all cases of CPT-II deficiency are currently detected through newborn screening, even when blood is appropriately collected on day 2 of life and (2) CPT-II deficiency should be kept on the differential for patients presenting with rhabdomyolysis, even if the newborn screening results were normal.

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Introduction

Patients with some inborn errors of metabolism, including long-chain fatty acid (LCFA) oxidation defects, can present with rhabdomyolysis, a clinical syndrome resulting from muscle breakdown (Chan et al. 2015). Carnitine palmitoyltransferase-II (CPT-II, EC 2.3.1.21) deficiency (OMIM: 255110) is one of the most common defects of LCFA metabolism (Bonnefont et al. 2004). CPT-II catalyzes formation of LCFA acyl-CoA species in mitochondria, allowing further oxidation and energy generation by other LCFA oxidation enzymes, including very-long-chain acyl-CoA dehydrogenase (VLCAD, EC 1.3.8.9) (Bonnefont et al. 2004). Patients with CPT-II deficiency show elevated blood levels of long-chain acylcarnitine species, especially palmitoyl-carnitine (C16) and oleoyl-carnitine (C18:1). CPT-II deficiency can be categorized as the myopathic form (OMIM: 255110), the early infantile hepatocardiomuscular form (OMIM: 600649), or the lethal neonatal form (OMIM: 608836) with multisystem involvement (Bonnefont et al 2004). Patients with the myopathic form present with rhabdomyolysis, which may be complicated by life-threatening events, including acute renal failure from myoglobinuria, respiratory insufficiency due to diaphragm involvement (Smolle et al. 2001), and paroxysmal cardiac arrhythmia (Thuillier et al. 2000).

Screening for fatty acid oxidation defects, including long- and medium-chain defects, is recommended in the USA in accordance with the guidelines of the US Secretary of Health and Human Services' Recommended Uniform Screening Panel (http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders /recommendedpanel/indes.html) and the American College of Medical Genetics Newborn Screening Expert Group (Watson et al. 2006). CPT-II deficiency is screened in almost all US newborn

Table 1 Acylcarnitine profile at birth (bloodspot) and on presentation (plasma) in a 4-year-old girl experiencing acute rhabdomyolysis

	Newborn screen, bloodspot (μmol/L)		On presentation, plasma (μmol/L)	
Acylcarnitine species	Value	Cutoff	Value	Normal range
Acetyl (C2)	13.13		9.12	4.21–20.60
Palmitoyl-carnitine (C16)	3.44	<10	2.2	<1
Oleoyl-carnitine (C18: 1)	1	<3	0.81	<0.5
Carnitine	14.5	≥5		
Linoleoyl-carnitine (C18: 2)			0.37	<0.3
Octadecanoyl-carnitine (C18:0)			0.39	<0.11
Dicarboxyoleyl-carnitine (C18: 1-DC)			0.05	<0.03

screening (NBS) programs as a secondary target of NBS due to the lack of a proven efficacious treatment (Watson et al. 2006). Although newborns are routinely screened for fatty acid oxidation defects, some cases of LCFA oxidation defects (Schymik et al. 2006; Ficicioglu et al. 2010; Sahai et al. 2011) may be missed on NBS. Here, we report a case of rhabdomyolysis due to CPT-II deficiency in a child with normal NBS results.

Case Report

A previously healthy and developmentally normal 4-year-old girl was admitted to our general pediatrics service with acute rhabdomyolysis. Her parents reported a 2-day history of fevers (T_{max} 39.4°C), diffuse myalgia, tea-colored urine, upper respiratory infection, and two episodes of nonbloody, nonbilious emesis. The patient's medical history was notable for frequent diffuse myalgia when ill. There was no known family history of musculoskeletal, renal, or metabolic disorders, and her parents were nonconsanguineous. Her birth history was unremarkable. She was exclusively breastfed in the neonatal period without concern for hypoglycemia. An NBS obtained on day-of-life 2 revealed normal acylcarnitine profile results (Table 1).

On examination, the patient was nondysmorphic and exhibited nasal congestion, diffuse muscle aches in her arms that worsened with palpation, and a full range of motion of all extremities. Her initial creatine kinase level was 21,000 U/L, which peaked at 74,000 U/L. Her urine was dark. Although minimal red blood cells were seen on microscopy, initial urinalysis detected the presence of blood, consistent with rhabdomyolysis. A plasma acylcarnitine profile showed elevated levels of long-chain acylcarnitine species, which suggested the possibility of CPT-II or carnitine-acylcarnitine translocase (CACT) deficiency (OMIM: 212138, Table 1). Further genetic testing showed a homozygous mutation c.338C>T, p. S113L in *CPT2* and no mutations in *SLC25A20* gene.

Discussion

Tandem mass spectrometry analysis of acylcarnitine species is sensitive and specific for most fatty acid oxidation disorders (Watson et al. 2006). The most sensitive indicator to detect CPT-II deficiency is an elevated (C16 + C18:1)/C2 ratio (Marquardt et al. 2012). However, a study of simultaneous analysis of plasma and dried blood spot (DBS) from individuals with known metabolic diagnoses suggested that this indicator can sometimes be unreliable because some patients' (C16 + C18:1)/C2 ratio may be normal in DBSs (de Sain-van der Velden et al. 2013). Our patient's (C16 + C18:1)/C2 ratio of 0.34 was 0.03 less than the control 95th percentile for DBSs reported in that paper (0.37). We analyzed our patient's NBS acylcarnitine values with the post-analytical tool available from the Laboratory Performance Database (R4S) (McHugh et al. 2011). This produced the following results: (1) our patient's (C16 + C18:1)/C2 ratio was under the 5th percentile for CPT-II deficiency and just over the 99th percentile for normal and classified in the "non-informative" range; (2) the case score was "zero"; and (3) the C3/C16 ratio was the only marker in the disease range (Fig. 1 and Table 2). In this case, tandem mass spectrometry failed to detect CPT-II deficiency.

There are several theoretical explanations for false-negative NBS results in disorders of fatty acid oxidation. These include (1) an anabolic state at the time of testing; (2) residual enzyme activity to overcome the catabolic stress of parturition; (3) depletion of carnitine, resulting in normalization of the long-chain acylcarnitine species; (4) excessively high cutoff values for acylcarnitine species that may disallow detection of all affected neonates; (5) sample mislabeling; and (6) laboratory error.

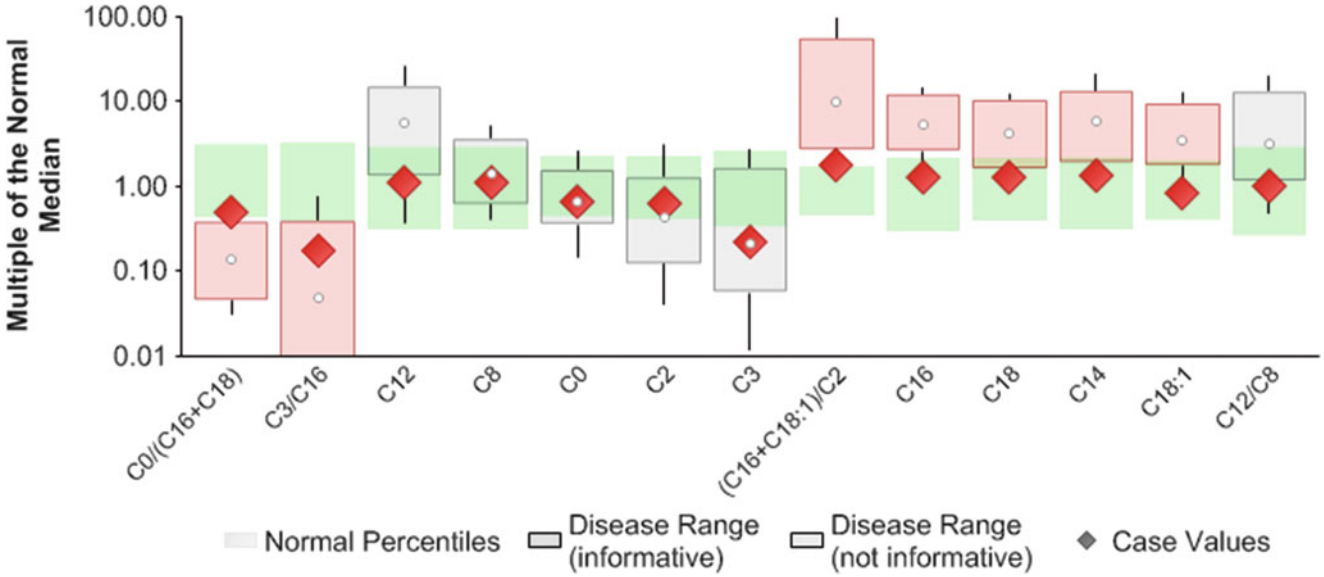

Fig. 1 Laboratory Performance Database (R4S) cumulative multiple of the normal median and CPT-II deficiency range overlap plot. Reported patient's NBS values are indicated with *red diamonds*

Table 2 Laboratory Performance Database (R4S): cumulative normal percentile and CPT-II disease range overlap values

Analyte	Normal 1%ile	Overlap %ile	Disease range 99%ile	95%ile	90%ile	50%ile	Case Values
CO/(C16+C18)	2.91	3.9%	4.86	2.72	2.51	0.91	3.24
C3/C16	0.25	8.9%	0.47	0.38	0.24	0.03	0.11
	99%ile	%ile	1%ile	5%ile	10%ile	50%ile	Values
C12	0.34	27.1%	0.04	0.12	0.16	0.68	0.13
C8	0.17	80.6%	0.03	0.03	0.04	0.09	0.07
C0	50.45	95.2%	3.28	5.75	8.37	14.79	14.53
C2	47.21	97.5%	0.86	1.85	2.68	8.90	13.13
C3	4.37	98.3%	0.02	0.05	0.10	0.38	0.38
(C16+C18:1)/C2	0.33	2.0%	0.25	0.39	0.55	1.98	0.34
C16	5.67	6.8%	2.37	5.23	7.33	14.01	3.44
C18	1.68	13.1%	0.86	1.05	1.35	3.23	1.04
C14	0.48	13.2%	0.26	0.40	0.44	1.31	0.30
C18:1	2.49	13.8%	0.90	1.87	2.31	4.45	1.05
C12/C8	5.33	46.1%	0.90	1.62	2.22	6.00	1.86

Reported case's values are in the last column
NP–DR Overlap
NP normal percentiles, *DR* disease range

The stress of parturition is thought to be sufficient to expose biochemical abnormalities by day-of-life 2. To our knowledge, this is the first report of missed CPT-II deficiency on NBS when the blood sample was appropriately collected on day 2 of life. One prior report of missed CPT-II deficiency on NBS was attributed to the establishment of adequate nutrition by day-of-life 5, when the NBS sample was obtained (Kobayashi et al. 2007). However, other reports of missed cases of LCFA oxidation defects, especially VLCAD deficiency (Schymik et al. 2006; Ficicioglu et al. 2010; Sahai et al. 2011), suggest that false-negative NBS results can occur even when blood is appropriately collected on day 2 of life (Ficicioglu et al. 2010).

Our patient had a homozygous mutation c.338C>T, p. S113L in *CPT2*, which is typically found in the adult-onset, myopathic form of the disease (Bonnefont et al. 2004). It is possible that the c.338C>T, p. S113L mutation has sufficient residual activity to overcome the catabolic stress of parturition and to result in a normal NBS profile. Our patient had a normal NBS carnitine level, making carnitine depletion an unlikely cause of the false-negative result. Her NBS values were significantly below current cutoffs, but lowering NBS cutoffs to capture her values would result in unacceptably high false-positive rates. The only abnormal marker was the C3/C16 value based on the R4S post-analytical tool (McHugh et al. 2011) (Fig. 1). The C3/C16 ratio may be used as a marker in the detection of mild CPT-II cases and should be considered in addition to C16 and (C16 + C18:1)/C2. However, more studies should be undertaken to assess if the application of a simple cutoff for this ratio would indeed increase sensitivity without negative impact on specificity and false-positive rate. It is unlikely that our patient's NBS sample was mislabeled because the screening laboratory did not report a corresponding false-positive result around the same time. Laboratory error is also possible but unlikely, as repeat acylcarnitine analysis showed normalization of the patient's profile when not ill. In a previous case report, a patient with demonstrated heterozygous novel variants in *CPT2* and a positive CPT-II deficiency result on NBS on day-of-life 3 showed normalization of the acylcarnitine profile on day-of-life 9 (Illsinger et al. 2008). Given that newborns have only undergone regular NBS capable of detecting CPT-II deficiency since the late 1990s, there may be other patients with normal NBS results who have not yet presented to medical care with the adult-onset myopathic form of the disease.

Rhabdomyolysis can result in severe hyperkalemia, hypocalcemia, hepatic inflammation (Sauret et al. 2002), and acute renal failure (ARF) (Mannix et al. 2006; Bosch et al. 2009). In pediatric patients, rhabdomyolysis is typically attributable to viral myositis in the first decade of life and to trauma or a drug-related pathology in the second decade of life (Mannix et al. 2006). Rhabdomyolysis in CPT-II deficiency is caused by the accumulation of toxic long-chain acylcarnitine species, usually resulting from prolonged exercise, but also occasionally from prolonged fasting, excess fat intake, cold exposure, fever, or certain drugs (e.g., valproic acid, diazepam, general anesthesia, and ibuprofen) (Bonnefont et al. 2004).

Treatment of CPT-II deficiency includes avoiding triggers of increased fatty acid oxidation to prevent the accumulation of long-chain acylcarnitine species and rhabdomyolysis and supplementing the diet with medium-chain triglycerides to bypass the metabolic blockade (Bonnefont et al. 2004). A formulation of odd-chain fatty acids (triheptanoin) is under investigation as an alternative therapy to medium-chain triglycerides (Vockley et al. 2015). In cases of concurrent rhabdomyolysis, aggressive fluid therapy is recommended to avoid ARF. Furthermore, high-dose intravenous dextrose can be used to reverse catabolism and stop the production of long-chain acylcarnitine species. These approaches to the treatment of CPT-II deficiency, including nonstandard approaches for the prevention and treatment of rhabdomyolysis, raise the question of whether it would be timely to consider CPT-II deficiency for inclusion in the core NBS panel rather than as a secondary target in the USA. In terms of the natural course of disease, phenotype, and treatment, CPT-II deficiency closely resembles VLCAD deficiency, which is included in the core panel.

We offer two conclusions. First, not all cases of CPT-II deficiency are currently detected through NBS, even when blood is appropriately collected on day 2 of life. Second, CPT-II deficiency should be kept on the differential for patients presenting with rhabdomyolysis, even if the NBS results were normal, because the treatments, complications, and recurrence rates of rhabdomyolysis in CPT-II-deficient patients differ from those of standard rhabdomyolysis.

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Synopsis

CPT-II deficiency should be kept on the differential for patients presenting with recurrent rhabdomyolysis, even if the newborn screening results were normal, because this disorder can be missed on newborn screening, and the treatments, complications, and recurrence rates of rhabdomyolysis in CPT-II-deficient patients differ from those of standard rhabdomyolysis.

Compliance with Ethics Guidelines

Conflict of Interest

Dr. Andrew Edmondson, Dr. Jennifer Salant, Dr. Lynne Ierardi-Curto, and Dr. Can Ficicioglu declare that they have no conflicts of interest.

Informed Consent/Animal Rights

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

Details of the Contributions of Individual Authors

Dr. Edmondson drafted the initial manuscript and revised the manuscript.

Dr. Salant contributed to drafting the initial manuscript.

Dr. Ierardi-Curto critically reviewed and revised the manuscript.

Dr. Ficicioglu critically reviewed and revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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