Available online at www.sciencedirect.com
ScienceDirect
Molecular Genetics and Metabolism 90 (2007) 441–445
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

Brief Communication

Expanded newborn screening identifies maternal primary carnitine deficiency

Lisa A. Schimmenti [a], Eric A. Crombez [b], Bernd C. Schwahn [c], Bryce A. Heese [d], Timothy C. Wood [e], Richard J. Schroer [e], Kristi Bentler [a], Stephen Cederbaum [b], Kiki Sarafoglou [a], Mark McCann [f], Piero Rinaldo [d], Dietrich Matern [d], Cristina Amat di San Filippo [g], Marzia Pasquali [g], Susan A. Berry [a,*], Nicola Longo [g,*]

[a] *University of Minnesota, Department of Pediatrics, Division of Genetics and Metabolism, Institute of Human Genetics, Minneapolis, MN, USA*
[b] *The David Geffen School of Medicine at UCLA, Department of Pediatrics, Division of Genetics and Metabolism, Los Angeles, CA, USA*
[c] *Department of General Pediatrics, University Children's Hospital Düsseldorf, Moorenstrasse 5, 40225 Düsseldorf, Germany*
[d] *Mayo Clinic College of Medicine, Department of Laboratory Medicine and Pathology, Division of Laboratory Genetics, Rochester, MN, USA*
[e] *Greenwood Genetic Center, Greenwood, SC, USA*
[f] *Minnesota Department of Health, Minneapolis, MN, USA*
[g] *University of Utah, Departments of Pediatrics, Pathology and ARUP Laboratories, Salt Lake City, UT, USA*

Received 16 October 2006; accepted 17 October 2006
Available online 28 November 2006

Abstract

Primary carnitine deficiency impairs fatty acid oxidation and can result in hypoglycemia, hepatic encephalopathy, cardiomyopathy and sudden death. We diagnosed primary carnitine deficiency in six unrelated women whose unaffected infants were identified with low free carnitine levels (C0) by newborn screening using tandem mass spectrometry. Given the lifetime risk of morbidity or sudden death, identification of adult patients with primary carnitine deficiency is an added benefit of expanded newborn screening programs.

Keywords: Carnitine deficiency; Newborn screening; Sudden death; Maternal disease; Carnitine transport; OCTN2

Introduction

Analysis of blood spots through tandem mass spectrometry has revolutionized newborn screening for inborn errors of metabolism. The simultaneous detection of multiple metabolites allows the presumptive identification of patients with inborn errors of amino acid, organic acid, and fatty acid metabolism within a few days after birth [1]. Early identification and prospective treatment can prevent catastrophic illness as a result of metabolic decompensation [2].

Primary carnitine deficiency (OMIM No. 212140) is an autosomal recessive disorder of fatty acid oxidation caused by mutations in the *SLC22A5* gene encoding the high-affinity carnitine transporter, OCTN2 [3–5]. Deficiency of this transporter increases urinary carnitine losses and produces tissue carnitine deficiency. Since carnitine is required for the entry of long-chain fatty acids into mitochondria, tissue carnitine deficiency impairs mitochondrial fatty acid β-oxidation with defective energy production during fasting [6]. Affected patients typically present in childhood with hypoketotic hypoglycemia, hepatic encephalopathy, hypotonia, cardiomyopathy or sudden death [6,7]. Diagnosis is based on the identification of very low free carnitine levels

* Corresponding authors. Fax: +1 612 626 2993 (S.A. Berry); fax: +1 801 587 7690 (N. Longo).
E-mail addresses: berry002@umn.edu (S.A. Berry), Nicola.Longo@hsc.utah.edu (N. Longo).

1096-7192/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.ymgme.2006.10.003

in plasma and is confirmed by the measurement of diminished carnitine transporter activity in skin fibroblasts [8] or mutational analysis of the *SLC22A5* gene [5,9]. This disorder responds well to oral carnitine supplementation (starting at 100 mg/kg/day) that can prevent crises and reverse cardiomyopathy if present [6,7].

Primary carnitine deficiency can be identified in infants by expanded newborn screening using tandem mass spectrometry by detection of low levels of free carnitine (C0) [3]. In affected infants, oral carnitine supplementation is followed by a slow normalization of plasma carnitine levels [6,7]. In the cases described herein, we observed a group of infants with low newborn screening free carnitine with a rapid rise in plasma levels upon supplementation. Our studies show that the observed infants' low carnitine levels resulted from primary carnitine deficiency in their affected mothers.

Materials and methods

Subjects

Mothers identified by a low free carnitine (C0) on newborn screening of their infants are reported in Table 1. All infants have been asymptomatic. The mothers of cases 2, 3 and 5 were asymptomatic. The mothers of cases 1 and 4 had decreased stamina, easy fatigability with exercise, and in one case fasting intolerance. All these symptoms have been reported in patients with primary carnitine deficiency [8,9]. Evaluation of their hearts by physical exam, echocardiography or electrocardiography did not reveal any anomaly.

The mother described as case 6, became symptomatic at 20 years of age. She suffered a syncopal episode and was diagnosed with prolonged QT interval causing ventricular tachycardia. A defibrillator was implanted. Over the following 5 years, she had two further episodes of tachycardia. During the first trimester of pregnancy, she had ten more events that activated automatic defibrillation. These events did not recur in the second part of pregnancy with modification of anti-arrhythmic therapy. She was diagnosed with carnitine deficiency after the birth of her child and placed on carnitine, with improvement of plasma carnitine levels. Cardiac function improved and there have been no new episodes of cardiac arrest since starting carnitine.

DNA analysis and molecular techniques

The protocol for DNA studies was approved by the University of Utah Institutional Review Board. Molecular testing for mutations in *SLC22A5* the gene encoding OCTN2 was performed after obtaining informed consent for DNA studies. GenBank sequence AB016625.1 was used as reference for the gene, NM_003060.2 was used as the reference sequence for the cDNA. Genomic DNA was extracted from fibroblasts or peripheral blood by standard methods and amplified using PCR and primers flanking each of the 10 exons [8,9]. Mutations were identified by bidirectional sequencing and confirmed by sequencing a second, independent PCR product.

Carnitine transport

All mothers underwent a skin biopsy for diagnostic testing of carnitine transport in fibroblasts. Fibroblasts were grown in Dulbecco-modified MEM supplemented with 15% fetal bovine serum. Carnitine (0.5 μM) transport was measured at 37 °C for 4 h as described previously [8,9]. This procedure assesses the initial rate of carnitine entry into the cells. Nonsaturable carnitine transport was measured in the presence of 2 mM cold carnitine and was subtracted from total transport to obtain saturable transport.

Table 1
Maternal carnitine deficiency identified by newborn screening

Maternal Cases (age in years, location)	Infant carnitine (C0) MS/MS newborn screen (μmo/L)	Infant plasma free carnitine on supplements (μmo/L)	Maternal plasma free carnitine (μmol/L)	Carnitine transport (percent of control)	*SLC22A5* mutations allele1(exon)/allele2 (exon)	Symptoms, cardiac evaluation
1. Mother (28, MN)	2.5	137	2	3.3	c.136 C > G, P46S (1)/ c.844 C > T, R282X (5)	Fasting intolerance, fatigue, normal echocardiogram
2. Mother (34, MN)	4.4	38	4	4.2	c.136 C > G, P46S (1)/ c.1556dupACAC T520fsX521 (9)	None, normal echocardiogram
3. Mother (30, D)	7.7	21	3	3.7	c.1462 C > T, R488C (9)/ c.1462 C > T, R488C (9)	None, normal echocardiogram
4. Mother (34, CA)	8	37	4	2	c.760 C > T, R254X (4)/ c.1400 C > G, S467C (8)	Decreased stamina, nonspecific T wave abnormality, normal echocardiogram
5. Mother (28, MN)	3.3	53	5	7.7	ND	None, normal echocardiogram
6. Mother (25, SC)	4.1	70.5	1.9	4.8	c.95A > G, N32S (1)/ c.136C > G, P46S (1)	Ventricular arrhythmias, prolonged QT interval

Carnitine levels were measured in newborn screening spots (second column) or in plasma (third and fourth column). Carnitine supplements were given orally at 25–100 mg/kg/day (third column). Normal levels for plasma free carnitine (third and fourth columns) are 25–50 μmol/L. Carnitine transport is expressed as percent of normal controls (fifth column). Mutations identified in the *SLC22A5* gene are reported in the sixth column. Symptoms and cardiac evaluation was performed by physical examination, electrocardiogram, and echocardiogram. ND, not done. MN, Minnesota; D, Germany; CA, California; SC, South Carolina.

Results

Over the course of 2 years, we identified a number of infants with very low free carnitine levels obtained at 24–48 h after birth by tandem mass spectrometry. Table 1 summarizes laboratory and clinical findings in six infants and their mothers. Five of the six mothers were of Caucasian origin and one mother was of Asian origin. In newborn screening blood spot samples, the infants had free carnitine (C0) levels between 2.5 and 8 μmol/L (column 2). Measurements of plasma carnitine levels at 7–21 days of life confirmed carnitine deficiency (free carnitine 5–9 μmol/L, normal 25–50 μmol/L). Typically, we observe a slow increase in plasma carnitine levels in patients with primary carnitine deficiency when carnitine supplements are started. In contrast these infants treated with carnitine (25–100 mg/kg/day) normalized free carnitine levels within weeks (column 3).

As part of the clinical evaluation, plasma carnitine levels were measured in the infants' mothers. The mothers of these infants (age 25–34 years) had very low plasma free carnitine levels (1.9–5 μmol/L, normal 25–50 μmol/L) (column 4), in the range observed in patients with primary carnitine deficiency. Carnitine is transferred from the placenta to the fetus during intrauterine life. Levels of free carnitine in infants reflect those of their mothers if the levels are obtained shortly after birth [10,11]. To confirm the suspected diagnosis of primary carnitine deficiency, skin biopsies were obtained to determine if the mothers had defective fibroblast carnitine transport.

Carnitine transport in fibroblasts from all the mothers tested was markedly impaired compared to normal cells (2–7.7% of normal) and to cells obtained from carriers for primary carnitine deficiency (Fig. 1, summary in Table 1, column 5). Mutation analysis of the *SLC22A5* gene encoding the OCTN2 carnitine transporter identified mutations in all samples tested (Table 1, column 6). Of the mutations identified, N32S, R254X, R282X, and S467C were previously reported in patients with classic primary carnitine deficiency [5,12–17]. The duplication of 4 bp in exon 9 of patient 2 producing a frameshift and the premature insertion of a stop codon (T520fsX521) is likely to result in nonsense mediated RNA decay, as reported in all other patients with primary carnitine deficiency with similar mutations [5,8] or in a severely truncated protein lacking the C-terminal 37 amino acids (truncation before position 526 of the OCTN2 transporter completely abolishes carnitine transport; Amat di San Filippo and Longo, unpublished results). The P46S missense mutation has not been previously reported. Expression of the P46S-mutant OCTN2 cDNA in Chinese Hamster Ovary cells decreased carnitine transport to <5% of that measured using normal OCTN2 cDNA, as seen with several other missense mutations associated with classic primary carnitine deficiency [8,9].

Based on clinical observation of low carnitine levels, impaired fibroblast carnitine transport activity and the identification of two pathogenic mutation in *SLC22A5*, we confirmed that the low carnitine levels in newborn screening blood spots resulted from previously undiagnosed maternal primary carnitine deficiency.

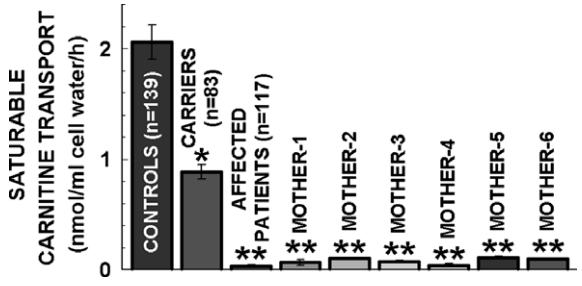

Fig. 1. Carnitine transport by fibroblasts from mothers with primary carnitine deficiency. Carnitine (0.5 μM) transport was measured for 4 h at 37 °C. Nonsaturable transport, measured in the presence of 2 mM cold carnitine, was subtracted from total transport to obtain saturable carnitine transport. Data are means ± SE of six observations for mothers' cells while the number of observations for normal controls, obligate carriers, and affected controls (patients with classic primary carnitine deficiency and demonstrated mutations) is indicated. *$p < 0.01$ versus controls, affected patients and mothers; **$p < 0.01$ versus control and carriers using analysis of variance.

Discussion

Primary carnitine deficiency is a life-threatening inborn error of metabolism. We show that this disorder can be suspected in mothers identified by the presence of low free carnitine (C0) on newborn screening of their unaffected infants (Table 1). In these infants, carnitine levels improved briskly on carnitine supplements (Table 1). The mothers of these infants had plasma carnitine levels similar to those of patients with classic primary carnitine deficiency (Table 1) and their diagnosis was confirmed by measuring carnitine transport in fibroblasts (Fig. 1) and/or by mutational analysis of the *SLC22A5* gene (Table 1). Some of the mutations identified (N32S, R254X, R282X, and S467C) have been found in other patients with classic primary carnitine deficiency, others (P46S, R488C, and T520fsX521) have not been described previously. Based on fibroblast and Chinese Hamster Ovary cells expression studies, these novel mutations severely impair carnitine transport [8,9].

Most affected mothers had minimal or no symptoms at time of diagnosis (Table 1). One, however, had a history of syncope that worsened during pregnancy when plasma carnitine levels are physiologically lower [18]. Further studies and a longer follow-up are required to define the relationship between carnitine deficiency and cardiac arrhythmia in this case. The other maternal cases indicate that a significant cohort of patients with primary carnitine deficiency do not present in infancy or early childhood as currently described [6,7], but remain asymptomatic into adulthood. Because other fatty acid oxidation defects such as medium chain acyl CoA dehydrogenase deficiency (MCADD) can remain completely asymptomatic until causing sudden

death or other acute presentation during severe stress in adults [19–21], we believe that mothers with primary carnitine deficiency are likely to benefit from diagnosis and initiation of carnitine supplementation. As we were preparing this manuscript for publication, Vijay et al. [22] reported four mothers from consanguineous Asian families with carnitine transporter defects identified through low free carnitine levels in their newborn infants. Our patients were of different ethnic backgrounds without consanguinity (except in case 3) and had different mutations in the carnitine transporter gene (Table 1, mutations were not reported in the previous cases, [22]) indicating that this disorder is not limited to specific ethnic groups. Primary carnitine deficiency has also been identified in the asymptomatic father of a patient with classic primary carnitine deficiency [23], indicating that males can also have the disease. This would be expected in an autosomal recessive disorder.

While the precise frequency of this disorder in adults is not known, the previous report identified four affected mothers in 62,004 infants screened, with a frequency of 1:15,500 [22]. Limiting our analysis to cases in Minnesota, four mothers (one not reported in Table 1) were identified out of approximately 161,420 births spanning the period of time from July 1, 2004 until September 30, 2006, providing an approximate frequency of 1:40,000. Since asymptomatic males are not identified by this system, this frequency likely underestimates the prevalence of this disorder in adults. A larger population is required to define the precise prevalence of this condition. In infants, the frequency reported is about 1:40,000, with about 1% of the population being a carrier for this disease [17].

With expanded newborn screening, we can identify maternal cases of 3-methylcrotonyl-CoA carboxylase deficiency (3-MCC) [24] or, through family studies of affected infants, MCAD [25]. Our cases, collected over the relatively short time period of 2 years, indicate that maternal primary carnitine deficiency can be detected by expanded newborn screening. We suspect that maternal primary carnitine deficiency goes unrecognized as the newborn screening low free carnitine levels may be considered to be false positives as confirmatory testing is usually limited to the infant. Our results support testing both the infant *and mother* in the event of low newborn screening carnitine levels. Primary carnitine deficiency carries a lifetime risk of sudden death; it is not a benign condition. The diagnoses of maternal primary carnitine deficiency in these six cases demonstrate that expanded newborn screening has the power to detect a cohort of hitherto asymptomatic adult individuals at risk for metabolic decompensation and possible sudden death.

Acknowledgment

Supported in part by Grant DK 53824 from the National Institutes of Health (N.L.).

References

[1] B. Wilcken, V. Wiley, J. Hammond, K. Carpenter, Screening newborns for inborn errors of metabolism by tandem mass spectrometry, N. Engl. J. Med. 348 (2003) 2304–2312.
[2] S.E. Waisbren, S. Albers, S. Amato, M. Ampola, T.G. Brewster, L. Demmer, R.B. Eaton, R. Greenstein, M. Korson, C. Larson, D. Marsden, M. Msall, E.W. Naylor, S. Pueschel, M. Seashore, V.E. Shih, H.L. Levy, Effect of expanded newborn screening for biochemical genetic disorders on child outcomes and parental stress, JAMA 290 (2003) 2564–2572.
[3] B. Wilcken, V. Wiley, K.G. Sim, K. Carpenter, Carnitine transporter defect diagnosed by newborn screening with electrospray tandem mass spectrometry, J. Pediatr. 138 (2001) 581–584.
[4] J. Nezu, I. Tamai, A. Oku, R. Ohashi, H. Yabuuchi, N. Hashimoto, H. Nikaido, Y. Sai, A. Koizumi, Y. Shoji, G. Takada, T. Matsuishi, M. Yoshino, H. Kato, T. Ohura, G. Tsujimoto, J. Hayakawa, M. Shimane, A. Tsuji, Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter, Nat. Genet. 21 (1999) 91–94.
[5] Y. Wang, J. Ye, V. Ganapathy, N. Longo, Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency, Proc. Natl. Acad. Sci. USA 96 (1999) 2356–2360.
[6] C.A. Stanley, Carnitine deficiency disorders in children, Ann. N. Y. Acad. Sci. 1033 (2004) 42–51.
[7] N. Longo, C. Amat Di San Filippo, M. Pasquali, Disorders of carnitine transport and the carnitine cycle, Am. J. Med. Genet. C Semin. Med. Genet. 142 (2006) 77–85.
[8] Y. Wang, S.H. Korman, J. Ye, J.J. Gargus, A. Gutman, F. Taroni, B. Garavaglia, N. Longo, Phenotype and genotype variation in primary carnitine deficiency, Genet. Med. 3 (2001) 387–392.
[9] C. Amat Di San Filippo, M. Pasquali, N. Longo, Pharmacological rescue of carnitine transport in primary carnitine deficiency, Hum. Mutat. 27 (2006) 513–523.
[10] F. Scaglia, N. Longo, Primary and secondary alterations of neonatal carnitine metabolism, Semin. Perinatol. 23 (1999) 152–161.
[11] J. Christodoulou, S.H. Teo, J. Hammond, K.G. Sim, B.Y. Hsu, C.A. Stanley, B. Watson, K.C. Lau, B. Wilcken, First prenatal diagnosis of the carnitine transporter defect, Am. J. Med. Genet. 66 (1996) 21–24.
[12] B. Burwinkel, J. Kreuder, S. Schweitzer, M. Vorgerd, K. Gempel, K.D. Gerbitz, M.W. Kilimann, Carnitine transporter OCTN2 mutations in systemic primary carnitine deficiency: a novel Arg169Gln mutation and a recurrent Arg282Ter mutation associated with an unconventional splicing abnormality, Biochem. Biophys. Res. Commun. 261 (1999) 484–487.
[13] E. Christensen, J. Holm, S.H. Hansen, N. Sorensen, J. Nezu, A. Tsuji, F. Skovby, Sudden infant death following pivampicilln treatment in a patient with carnitine transporter deficiency, J. Inherit. Metab. Dis. 23 (suppl. 1) (2000) 117.
[14] A.M. Lamhonwah, S.E. Olpin, R.J. Pollitt, C. Vianey-Saban, P. Divry, N. Guffon, G.T. Besley, R. Onizuka, L.J. De Meirleir, L. Cvitanovic-Sojat, I. Baric, C. Dionisi-Vici, K. Fumic, M. Maradin, I. Tein, Novel OCTN2 mutations: no genotype-phenotype correlations: early carnitine therapy prevents cardiomyopathy, Am. J. Med. Genet. 111 (2002) 271–284.
[15] N.L. Tang, W.L. Hwu, R.T. Chan, L.K. Law, L.M. Fung, W.M. Zhang, A founder mutation (R254X) of SLC22A5 (OCTN2) in chinese primary carnitine deficiency patients, Hum. Mutat. 20 (2002) 232.
[16] F.M. Vaz, H.R. Scholte, J. Ruiter, L.M. Hussaarts-Odijk, R.R. Pereira, S. Schweitzer, J.B. De Klerk, H.R. Waterham, R.J. Wanders, Identification of two novel mutations in OCTN2 of three patients with systemic carnitine deficiency, Hum. Genet. 105 (1999) 157–161.
[17] A. Koizumi, J. Nozaki, T. Ohura, T. Kayo, Y. Wada, J. Nezu, R. Ohashi, I. Tamai, Y. Shoji, G. Takada, S. Kibira, T. Matsuishi, A. Tsuji, Genetic epidemiology of the carnitine transporter OCTN2 gene in a japanese population and phenotypic characterization in japanese

pedigrees with primary systemic carnitine deficiency, Hum. Mol. Genet. 8 (1999) 2247–2254.
[18] M. Schoderbeck, B. Auer, E. Legenstein, H. Genger, P. Sevelda, H. Salzer, R. Marz, A. Lohninger, Pregnancy-related changes of carnitine and acylcarnitine concentrations of plasma and erythrocytes, J. Perinat. Med. 23 (1995) 477–485.
[19] F. Feillet, G. Steinmann, C. Vianey-Saban, C. De Chillou, N. Sadoul, E. Lefebvre, M. Vidailhet, P.E. Bollaert, Adult presentation of MCAD deficiency revealed by coma and severe arrythmias, Intensive Care Med. 29 (2003) 1594–1597.
[20] K. Raymond, A.E. Bale, C.A. Barnes, P. Rinaldo, Medium-chain acyl-CoA dehydrogenase deficiency: sudden and unexpected death of a 45 year old woman, Genet. Med. 1 (1999) 293–294.
[21] W. Ruitenbeek, P.J. Poels, D.M. Turnbull, B. Garavaglia, R.A. Chalmers, R.W. Taylor, F.J. Gabreels, Rhabdomyolysis and acute encephalopathy in late onset medium chain acyl-CoA dehydrogenase deficiency, J. Neurol. Neurosurg. Psychiatry 58 (1995) 209–214.
[22] S. Vijay, A. Patterson, S. Olpin, M.J. Henderson, S. Clark, C. Day, G. Savill, J.H. Walter, Carnitine transporter defect: diagnosis in asymptomatic adult women following analysis of acylcarnitines in their newborn infants, J. Inherit. Metab. Dis. 29 (2006) 627–630.
[23] U. Spiekerkoetter, G. Huener, T. Baykal, M. Demirkol, M. Duran, R. Wanders, J. Nezu, E. Mayatepek, Silent and symptomatic primary carnitine deficiency within the same family due to identical mutations in the organic cation/carnitine transporter OCTN2, J. Inherit. Metab. Dis. 26 (2003) 613–615.
[24] D.D. Koeberl, D.S. Millington, W.E. Smith, S.D. Weavil, J. Muenzer, S.E. Mccandless, P.S. Kishnani, M.T. Mcdonald, S. Chaing, A. Boney, E. Moore, D.M. Frazier, Evaluation of 3-methylcrotonyl-CoA carboxylase deficiency detected by tandem mass spectrometry newborn screening, J. Inherit. Metab. Dis. 26 (2003) 25–35.
[25] T.G. Derks, H. Jakobs, A. Gerding, K.E. Niezen-Koning, D.J. Reijngoud, G.P. Smit, deficiency of the fatty-acid oxidising enzyme medium-chain acyl-coA dehydrogenase (MCAD) in an adult, detected during a neonatal screening programme, Ned. Tijdschr. Geneeskd. 148 (2004) 2185–2190.