Carnitine Deficiency Disorders in Pediatrics

Annals of Nutrition & Metabolism

Ann Nutr Metab 2016;68(suppl 3):5–9
DOI: 10.1159/000448321

Published online: December 9, 2016

Primary Carnitine Deficiency and Newborn Screening for Disorders of the Carnitine Cycle

Nicola Longo

Department of Pediatrics and Pathology, ARUP Laboratories, University of Utah, Salt Lake City, Utah, USA

Key Words
Carnitine · Biosynthesis · Transport · Deficiency · Newborn screening · Genetic variations · Autism

Abstract
Carnitine is needed for transfer of long-chain fatty acids across the inner mitochondrial membrane for subsequent β-oxidation. Carnitine can be synthesized by the body and is also obtained in the diet through consumption of meat and dairy products. Defects in carnitine transport such as those caused by defective activity of the OCTN2 transporter encoded by the *SLC22A5* gene result in primary carnitine deficiency, and newborn screening programmes can identify patients at risk for this condition before irreversible damage. Initial biochemical diagnosis can be confirmed through molecular testing, although direct study of carnitine transport in fibroblasts is very useful to confirm or exclude primary carnitine deficiency in individuals with genetic variations of unknown clinical significance or who continue to have low levels of carnitine despite negative molecular analyses. Genetic defects in carnitine biosynthesis do not generally result in low plasma levels of carnitine. However, deletion of the trimethyllysine hydroxylase gene, a key gene in carnitine biosynthesis, has been associated with non-dysmorphic autism. Thus, new roles for carnitine are emerging that are unrelated to classic inborn errors of metabolism.

Carnitine is essential for the transfer of long-chain fatty acids across the inner mitochondrial membrane for subsequent β-oxidation [1]. Carnitine can be synthesized by the body and can be obtained in the diet by consumption of meat and dairy products. As such, genetic defects in carnitine biosynthesis do not routinely result in low plasma carnitine levels.

The Carnitine Cycle and Carnitine Deficiency in Newborns

Carnitine is accumulated in cells and retained by the kidneys via OCTN2, a high affinity organic cation transporter that is specific for carnitine [1]. Genetic defects in the OCTN2 carnitine transporter result in primary carnitine deficiency, which is associated with decreased accumulation of intracellular carnitine, in-

KARGER

E-Mail karger@karger.com
www.karger.com/anm

0250–6807/16/0687–0005$39.50/0

Prof. Nicola Longo
Department of Pediatrics and Pathology, ARUP Laboratories
University of Utah, 295 Chipeta Way
Salt Lake City, UT 84108 (USA)
E-Mail Nicola.Longo@hsc.utah.edu

creased loss of carnitine in urine and low levels of carnitine in serum. Affected individuals can present with hypoketotic hypoglycemia and hepatic encephalopathy early in life, or with skeletal and cardiac myopathy or sudden death from cardiac arrhythmia later in life, usually triggered by fasting or catabolic state. While defects in OCTN2 can lead to carnitine deficiency, other conditions have also been identified that can cause carnitine deficiency in newborns. These include dietary carnitine deficiency, total parenteral nutrition without added carnitine [2], maternal glutaric acidemia type I [3], maternal medium chain acyl coenzyme A (CoA) dehydrogenase deficiency [4], 3-methyl-crotonyl-CoA carboxylase deficiency [5]. There are also other conditions not associated directly with carnitine deficiency, that may warrant treatment with carnitine such as deletions in the trimethyllysine hydroxylase (*TMLHE*) gene on Xq28 encoding for ε-N-TMLHE, a risk factor for non-syndromic autism-spectrum disorders in males [6, 7].

Carnitine transporter deficiency
(Primary carnitine deficiency MIM 212140)

- ***Frequency***: 1:142,000 (USA), 1:40,000 (Japan), 1:300 (Faroe Islands)
- ***Cause***: Carnitine transporter (OCTN2) defect (*SLC22A5* gene)
- ***Pathogenesis***: Loss of carnitine in urine reduces availability of carnitine in liver, muscle and heart, impairing fatty acid oxidation
- ***Presentation***: Hepatic encephalopathy, hypoglycemia, cardiomyopathy in childhood, arrhythmia in adults, sudden death in children and adults
- ***Diagnosis***: Plasma carnitine levels (very low, usually <5 μM, can be higher in newborns), decreased urinary carnitine reabsorption, confirmed by transport studies in fibroblasts or DNA testing. Can be detected by newborn screening
- ***Therapy***: Carnitine 100–150 mg/kg up to 3 g per day PO divided into 3–4 daily doses
- ***Monitoring***: Plasma carnitine free and total
- ***Prognosis***: Excellent with treatment

Fig. 1. Key features of primary carnitine deficiency.

The Need to Screen for and Treat Primary Carnitine Deficiency

Primary carnitine deficiency responds to oral carnitine that, at pharmacological doses, enters cells using the amino acid transporter $B^{0,+}$ [1]. Key aspects of carnitine transporter deficiency are shown in figure 1.

The incidence of primary carnitine deficiency varies with a frequency of approximately 1:40,000 newborns in Japan [8], 1:37,000–1:100,000 newborns in Australia [9] and 1:142,000 in the USA [10]. The highest incidence (1:300) has been reported in the Faroe Islands, an archipelago that remained geographically isolated for centuries [11]. Decreased intracellular carnitine accumulation results in impaired fatty acid oxidation, and if carnitine supplements are not initiated, patients with primary carnitine deficiency can present with early acute metabolic decompensation, or later in life with skeletal and cardiac myopathy or sudden death from arrhythmia [12]. Newborn screening programmes for primary carnitine deficiency can identify affected patients at risk for this condition before irreversible damage occurs. Diagnosis of primary carnitine deficiency can be biochemically confirmed by demonstrating low free carnitine levels in plasma (<8 μM, normal 25–50 μM) with reduced renal reabsorption (<90%) and normal renal function with no abnormalities in urine organic acids [1]. Since a maternal disorder could also be responsible for primary carnitine deficiency, plasma and urine carnitine, plasma acylcarnitine profile and urine organic acids should be evaluated in the mother. The diagnosis is definitively confirmed by molecular testing of the *SLC22A5* gene or by studying carnitine transport in fibroblasts (<20% of normal controls).

Evaluation of Carnitine Transport in Fibroblasts

A functional assay (carnitine transport in fibroblasts) is very useful to confirm or exclude primary carnitine deficiency in individuals with genetic variations of unknown clinical significance or who continue to have low levels of carnitine despite negative molecular analyses. In addition, the parents of affected children have around half-normal carnitine transport in fibroblasts and may have low levels of plasma carnitine [13]. Functional studies in fibroblasts can also be considered as the most definitive test, since up to 16% of mutant alleles causing primary carnitine deficiency can not be identified by sequencing and deletion/duplication analysis of all 10 exons of the *SLC22A5* gene and flanking regions. Most patients have at least one missense mutation in the OCTN2 carnitine transporter and expression studies in Chinese hamster ovary (CHO) cells can confirm a causal role for single amino acid substitutions. Figure 2 shows that not all missense changes identified in patients with primary carnitine deficiency impair carnitine transport since some variations have the same activity of the wild type OCTN2.

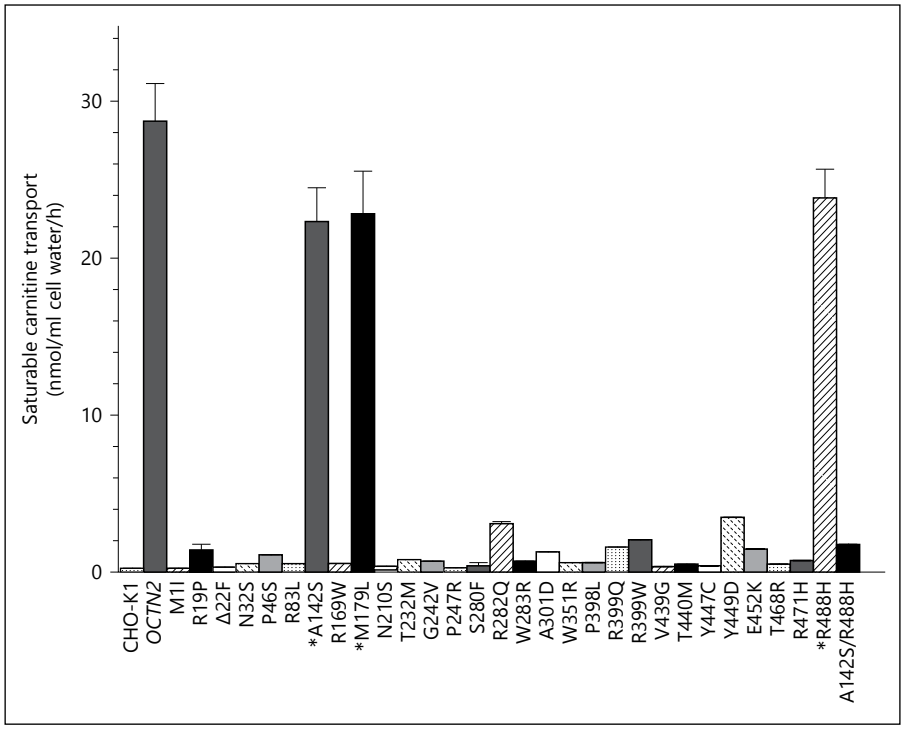

Fig. 2. Carnitine transport by CHO cells expressing normal and mutant OCTN2 carnitine transporters.

Shortcomings of Newborn Screening

A recent study by Therrell et al. [10] found that the frequency of primary carnitine deficiency among newborn screening programs in the USA is 1:142,000. However, the frequency of mutations (nonsense, splicing and expressed missense only) in 60,000 normal individuals (carriers) in the exac browser (http://exac.broadinstitute.org/gene/ENSG00000197375) is 1:141, with an extrapolated frequency of affected individuals (homozygous or compound heterozygous) of 1 in 79,910. Since the reported frequency is 1 in 142,236, this would suggest that many cases are missed by newborn screening. The possibility that newborn screening might miss cases of carnitine transporter deficiency should be considered in patients with persistent low or borderline levels of carnitine or who show a decrease in carnitine levels after stopping supplementation. Such individuals should be investigated for primary carnitine deficiency.

Strategies for Newborn Screening

There are different strategies for newborn screening that vary among states in the USA. In Utah, we perform 2 screenings, the first within about 48 h after birth, and the second at 7–21 days of life. Carnitine is transferred from the mother to the child via the placenta and levels of carnitine can be normal in an infant if the sample is collected shortly after birth. In this case, carnitine levels may decline over time. A two-step screening might allow better detection of infants with primary carnitine deficiency. On the other hand, maternal primary carnitine deficiency is better identified with very low carnitine levels on the first screening. In such cases, most mothers are symptomatic, but are at risk of sudden death. Carnitine supplementation in asymptomatic mothers can increase plasma carnitine levels and prevent cardiac arrhythmia.

Differences in Presentation of Primary Carnitine Deficiency

Unfortunately, there is little correlation between the type of mutation and timing or type (metabolic versus cardiomyopathy) of presentation in children with primary carnitine deficiency. Moreover, even within the same family, there is variability in clinical presentation, with some children presenting early with hepatic encephalopathy and others presenting later with cardiac dysfunction. This lack of genotype–phenotype correlation has been demonstrated in several studies [14–16].

This aspect has been studied in greater depth by Rose et al. [17] where carnitine transport was found to be significantly reduced in fibroblasts from all patients with primary carnitine deficiency, but significantly higher in fibroblasts from asymptomatic women. DNA sequence analysis found an increased frequency of nonsense mutations in symptomatic patients. Expression of missense mutations from asymptomatic patients in CHO cells indicated that many retained residual carnitine transport activity, with a decrease in the average activity of missense mutations identified in symptomatic versus asymptomatic patients. From these results, it was suggested that asymptomatic women have average levels of residual carnitine transport activity that are higher than symptomatic patients due to the presence of at least one missense mutation. However, not all mothers identified by newborn screening are asymptomatic. Chen et al. [18] reported on cases of carnitine uptake defects in 13 mothers identified by a newborn screening programme. One mother had cardiomyopathy, and another died suddenly 1 year after delivering. This latter individual had experienced a few episodes of syncope since 13 years of age without understanding the etiology, and diagnosis was made retrospectively. This highlights the need for screening in both infants and mothers.

The Faroe Islands: A Case Study

As mentioned above, the incidence of primary carnitine deficiency is very high in the Faroe Islands. This is a small archipelago in the North Atlantic located between Scotland and Iceland. The Faroe Islands are geographically isolated and are part of the Kingdom of Denmark, along with Denmark proper and Greenland. The population of the Faroe Islands is about 50,000 with another 20,000 living abroad, mostly in Denmark. It originated mainly from colonization by a small number of Norwegians about 1,000 years ago. The native population of the Faroe Islands has a very high incidence of several genetic conditions: cystic fibrosis, glycogen storage disease type IIIA, holocarboxylase synthase deficiency and 3-methylcrotonyl CoA carboxylase deficiency. The high incidence of primary carnitine deficiency is due to a founder mutation (p.N32S) [19]. Of interest, young adults (age 25–30 years) frequently died from ventricular fibrillation in the absence of cardiomyopathy. Some individuals also complained of lassitude and weakness, which improved with carnitine supplementation.

The *TMLHE* Gene, Carnitine Biosynthesis and Autism

Defects in carnitine synthesis are not responsible for carnitine deficiency since most carnitine is supplied through diet. However, deletion of the *TMLHE* gene, which is part of the carnitine synthesis pathway and located on the X chromosome, is found more often in males with non-dysmorphic autism [20]. In the study by Celestino-Soper et al. [20], *TMLHE* deficiency was found to be common in control males (1 in 366) and was not increased in frequency in probands from simplex autism families (1 in 323). However, it was nearly 3-fold more frequent in probands from male–male multiplex autism families compared with controls (1 in 130). Moreover, 6 of 7 autistic male siblings of probands in male–male multiplex families had the deletion, thus suggesting that *TMLHE* deficiency is a risk factor for autism, albeit with low penetrance (estimated at 2–4%). Hemizygous deletion of a second gene in the carnitine biosynthesis pathway, BBOX1, also results in low-normal carnitine levels, although it is unclear if the observed phenotypic effects (microcephaly and delays) are directly related to the decreased carnitine levels or to deletion of nearby genes [21]. The possible dysregulation of carnitine metabolism in non-dysmorphic autism thus warrants further study.

Conclusions

- OCTN2 carnitine transporter deficiency causes a spectrum of diseases, from hepatic encephalopathy to cardiomyopathy and sudden death.
- Newborn screening can miss infants with primary carnitine deficiency, in part due to the timing of the screening.
- DNA testing identifies about 85% of causative mutations, with the rest being in non-exonic regions.
- Defects in carnitine biosynthesis have been identified in humans, which results in low-normal carnitine levels and accumulation of intermediates before metabolic bloc – such defects might represent a risk factor for non-dysmorphic autism.
- New roles for carnitine are emerging in metabolic disorders that are unrelated to classic inborn errors of metabolism.

Disclosure Statement

N.L. received travel sponsorship and honorarium from SigmaTau to attend the carnitine symposium.

References

1 Longo N, Frigeni M, Pasquali M: Carnitine transport and fatty acid oxidation. Biochim Biophys Acta 2016;pii:S0167-4889(16)30013-1.
2 Borum PR: Carnitine in parenteral nutrition. Gastroenterology 2009;137(5 suppl):S129–S134.
3 Hedlund GL, Longo N, Pasquali M: Glutaric acidemia type 1. Am J Med Genet C Semin Med Genet 2006;142C:86–94.
4 Rhead WJ: Newborn screening for medium-chain acyl-CoA dehydrogenase deficiency: a global perspective. J Inherit Metab Dis 2006; 29:370–377.
5 Arnold GL, Salazar D, Neidich JA, Suwannarat P, Graham BH, Lichter-Konecki U, et al: Outcome of infants diagnosed with 3-methylcrotonyl-CoA-carboxylase deficiency by newborn screening. Mol Genet Metab 2012; 106:439–441.
6 Nava C, Lamari F, Heron D, Mignot C, Rastetter A, Keren B, et al: Analysis of the chromosome X exome in patients with autism spectrum disorders identified novel candidate genes, including TMLHE. Transl Psychiatry 2012;2:e179.
7 Ziats MN, Comeaux MS, Yang Y, Scaglia F, Elsea SH, Sun Q, et al: Improvement of regressive autism symptoms in a child with TMLHE deficiency following carnitine supplementation. Am J Med Genet A 2015;167A:2162–2167.
8 Koizumi A, Nozaki J, Ohura T, Kayo T, Wada Y, Nezu J, et al: 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 1999;8:2247–2254.
9 Wilcken B, Wiley V, Sim KG, Carpenter K: Carnitine transporter defect diagnosed by newborn screening with electrospray tandem mass spectrometry. J Pediatr 2001;138:581–584.
10 Therrell BL Jr, Lloyd-Puryear MA, Camp KM, Mann MY: Inborn errors of metabolism identified via newborn screening: ten-year incidence data and costs of nutritional interventions for research agenda planning. Mol Genet Metab 2014;113:14–26.
11 Rasmussen J, Nielsen OW, Janzen N, Duno M, Gislason H, Kober L, et al: Carnitine levels in 26,462 individuals from the nationwide screening program for primary carnitine deficiency in the Faroe Islands. J Inherit Metab Dis 2014;37:215–222.
12 Wang Y, Ye J, Ganapathy V, Longo N: Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. Proc Natl Acad Sci U S A 1999;96:2356–2360.
13 Scaglia F, Wang Y, Singh RH, Dembure PP, Pasquali M, Fernhoff PM, et al: Defective urinary carnitine transport in heterozygotes for primary carnitine deficiency. Genet Med 1998;1:34–39.
14 Lamhonwah AM, Olpin SE, Pollitt RJ, Vianey-Saban C, Divry P, Guffon N, et al: Novel OCTN2 mutations: no genotype-phenotype correlations: early carnitine therapy prevents cardiomyopathy. Am J Med Genet 2002;111: 271–284.
15 Wang Y, Korman SH, Ye J, Gargus JJ, Gutman A, Taroni F, et al: Phenotype and genotype variation in primary carnitine deficiency. Genet Med 2001;3:387–392.
16 Wang Y, Taroni F, Garavaglia B, Longo N: Functional analysis of mutations in the OCTN2 transporter causing primary carnitine deficiency: lack of genotype-phenotype correlation. Hum Mutat 2000;16:401–407.
17 Rose EC, di San Filippo CA, Ndukwe Erlingsson UC, Ardon O, Pasquali M, Longo N: Genotype-phenotype correlation in primary carnitine deficiency. Hum Mutat 2012;33:118–123.
18 Chen YC, Chien YH, Chen PW, Leung-Sang Tang N, Chiu PC, Hwu WL, et al: Carnitine uptake defect (primary carnitine deficiency): risk in genotype-phenotype correlation. Hum Mutat 2013;34:655.
19 Lund AM, Joensen F, Hougaard DM, Jensen LK, Christensen E, Christensen M, et al: Carnitine transporter and holocarboxylase synthetase deficiencies in the Faroe Islands. J Inherit Metab Dis 2007;30:341–349.
20 Celestino-Soper PB, Violante S, Crawford EL, Luo R, Lionel AC, Delaby E, et al: A common X-linked inborn error of carnitine biosynthesis may be a risk factor for nondysmorphic autism. Proc Natl Acad Sci U S A 2012;109: 7974–7981.
21 Rashidi-Nezhad A, Talebi S, Saebnouri H, Akrami SM, Reymond A: The effect of homozygous deletion of the BBOX1 and Fibin genes on carnitine level and acyl carnitine profile. BMC Med Genet 2014;15:75.