ND 29260

ACADEMIC PRESS

Molecular Genetics and Metabolism 77 (2002) 195–201

Molecular Genetics and Metabolism

www.academicpress.com

Carnitine membrane transporter deficiency: a long-term follow up and OCTN2 mutation in the first documented case of primary carnitine deficiency

Stephen D. Cederbaum,[a,b,c,d,*] Samantha Koo-McCoy,[e] Ingrid Tein,[h] Betty Y.L. Hsu,[e] Arupa Ganguly,[g] Eric Vilain,[b,c,d] Katrina Dipple,[b,d] Ljerka Cvitanovic-Sojat,[i] and Charles Stanley[e,f]

[a] *Department of Psychiatry, UCLA, 760 Westwood Plaza, Los Angeles, CA 90024-1759, USA*
[b] *Department of Pediatrics, UCLA, 760 Westwood Plaza, Los Angeles, CA 90024-1759, USA*
[c] *Department of Human Genetics, UCLA, 760 Westwood Plaza, Los Angeles, CA 90024-1759, USA*
[d] *Mental Retardation Research Center (MRRC), 760 Westwood Plaza, Los Angeles, CA 90024-1759, USA*
[e] *Division of Endocrinology, Childrens Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA, USA*
[f] *Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA*
[g] *Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA USA*
[h] *Department of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto, Ont., Canada*
[i] *Division of Neurology, Department of Pediatrics, University Hospital Sestre Milosrdnice, Zagreb, Croatia*

Received 3 September 2002; accepted 3 September 2002

Abstract

Three older patients were diagnosed with systemic carnitine deficiency in childhood nearly a generation ago and have together been treated for more than 50 patient years. Treatment improved tissue carnitine stores (proven in two) and eliminated most of the signs and symptoms of carnitine deficiency. All three have continued to respond to carnitine therapy and remain well except for the irreversible *sequelae* of the pretreatment illnesses. We demonstrate here that transformed lymphocytes from the first documented case of plasma membrane carnitine transporter deficiency fail to take up carnitine from the medium. The analysis of the cDNA of this patient and his parents revealed a homozygous frameshift mutation, 1027delT in exon 4. The resulting polypeptide terminates after amino acid 295. His parents are heterozygous for this mutation. The deletion resulted in predominately abnormal mRNA splicing with either a 13 or 19 bp insertion between the junction of exons 3 and 4. The 13/19 bp insertions were found in both parents, predominantly in cis with the deletion, and rarely seen with normal alleles from either parents or controls.
© 2002 Elsevier Science (USA). All rights reserved.

Keywords: Systemic carnitine deficiency; Carnitine transporter deficiency; Fatty acid oxidation defect

1. Introduction

The high affinity carnitine transporter defect is associated with severely reduced transport of carnitine into kidney, heart, and muscle, leading to an impairment of fatty acid oxidation. The defect is inherited in an autosomal recessive manner. The gene responsible has been recently cloned [1,2] and mapped to chromosome 5q31 [3]. It consists of 10 exons [1]. Systemic carnitine deficiency due to mutations in the OCTN2 gene results in recurrent hypoketotic hypoglycemia and/or dilated cardiomyopathy in patients [4–15].

In 1980 we published a report of a patient with systemic carnitine deficiency who had responded well to oral carnitine supplementation [4]. He ceased to have episodes of hypoglycemia, lethargy, and coma and he experienced no further hospital admissions. His large cardiac size returned to normal. We demonstrated

* Corresponding author. Fax: 1-310-206-5431.
E-mail address: scederbaum@mednet.ucla.edu (S.D. Cederbaum).

1096-7192/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
PII: S1096-7192(02)00169-5

carnitine depletion in blood, muscle, and liver and its repletion after months of oral therapy. Four years later we reported his continued good health and response to carnitine [16]. At that time we showed that ketogenesis, previously absent after a 32 h fast, had become substantially normal.

In our first report we had assumed that the high urinary excretion of carnitine reflected high absorption in the GI tract. We now know that this was a manifestation of the primary pathological feature of the defect, the deficiency of facilitated carnitine transport into heart and skeletal muscle, and its defective reabsorption in the kidney [17]. Subsequently this transport deficiency was demonstrated in cultured skin fibroblasts [18], transformed lymphocytes [19] and cultured myoblasts [20] from affected patients.

A similar clinical course and successful response to treatment is the normal experience with most, if not all, patients with this disorder, provided there is compliance with carnitine therapy [21].

We now report the follow-up of this patient for nearly a generation from initial diagnosis and treatment and confirm the supposition that this well studied patient does indeed have mutations in the carnitine transporter gene and defective uptake of carnitine into immortalized cultured lymphocytes. We also report the long-term follow-up of two more patients, demonstrating that all three have continued in stable good health on high dose oral carnitine supplementation, without any evidence of progressive cardiac or liver dysfunction.

2. Materials and methods

2.1. Case reports

Case 1. This boy, the offspring of non-consanguineous Mexican parents, became symptomatic at age 3 months and during the subsequent 3 years spent one-third of his life in UCLA during 11 hospital admissions. A brother died at age 3 months of liver disease, in Mexico. His father recently died from what was diagnosed as alcohol-related cirrhosis. The index case suffered eight episodes of cardiac arrest and had repeated episodes of severe hypoglycemia. Brain imaging revealed marked enlargement of both lateral ventricles and atrophy of the cerebral gyri. He had a severe hypertrophic cardiomyopathy.

With carnitine therapy, titrated to maintain plasma carnitine in the low to mid normal range (now 3 g–3 times/day), his cardiac function returned to normal and he required no hospital admissions for more than 20 years. He has weathered acute infections and high fevers without acute metabolic decompensation. Now at 26 years of age he is able to perform janitorial work with coaching and is gainfully employed. He is able to read, write and converse at a simple level and can get around by public transit as long as the route is familiar. His physical examination is normal except for mild truncal hypotonia (a persistent postural idiosyncrasy of his head tending to hang forward). Muscle strength and peripheral tone are normal as are his deep tendon reflexes. He is a fully grown adult, and has a normal weight and head circumference. His heart rate is normal and his pulse is strong. Blood pressure is normal. Cardiac size is normal and the heart sounds are full. An echocardiogram is entirely normal. There is no hepatomegaly.

At age 20 he suffered several episodes of staring and confusion that were consistent with partial complex seizures. An EEG was normal. The episodes have not recurred on tegretol therapy.

Case 2. This girl was born to non-consanguineous East Indian and Caucasian parents. She has been reported previously [22]. By 14 months of age she demonstrated a plateau of her gross motor milestones. Over the next 2 years, she developed slowly progressive weakness and recurrent respiratory tract infections. She suffered no documented episodes of hypoglycemic coma. At 3 years of age her clinical findings included congestive heart failure with moderate-to-severe mitral valve regurgitation. Echocardiography demonstrated marked left ventricular dilatation. At 4 years 3 months of age, she was found to have very low carnitine concentrations in serum and muscle. Her serum carnitine concentration was 19 μmol/L total, with a free carnitine of 15 μmol/L (controls: total 51.5 ± 11.6; free 40.0 ± 9.5). The muscle carnitine concentration was total/free: 0.17/0.11 μmol/g wet weight (controls: total/free $= 3.61/2.69 \pm 0.56$). The muscle carnitine was comparable to patient 1, whereas that in serum was higher. Carnitine uptake in cultured skin fibroblasts at an extracellular carnitine concentration of 5 μmol/L was 2% of normal controls.

On treatment with carnitine (doses adjusted to maintain carnitine in the low normal range), she had rapid subjective and objective improvement. Her exercise tolerance improved markedly and her weight increased from the 3rd to the 50th centile. Her markedly increased left ventricular dimensions returned toward normal, but remained mildly dilated. By age 9 years she was a competitive swimmer at school, had a high IQ, but continued to have mild hypotonia and a mildly myopathic facies. By age 22 she still had mild abnormalities in left ventricular size, but normal left ventricular function on echocardiography and was living a physically active life and studying at the University.

Case 3. This girl of Croation origin was born to healthy, non-consanguineous parents. She, too, has been reported previously [22]. She had normal early growth, but had always been considered to be hypotonic. At 2.5 years of age, during an episode of pharyngitis, she became flaccid and somnolent and on hospitalization, was found to have hypoglycemia (1 mmol/L), elevated liver

transaminases, and normal ammonia. An EEG revealed large amplitude slow waves. She returned to normal and had a normal serum glucose after 24 h. She suffered similar episodes 6 months and 1 year later in the context of viral infections and fever, which were accompanied by non-ketotic hypoglycemia. Echocardiography revealed a cardiomyopathy. A 24-h fasting test resulted in marked hypoglycemia and somnolence that lasted 3 days despite a continuous infusion of glucose. During a separate 17-h fast, urine organic acids were normal. Her free serum carnitine was below the level of detection, whereas acylcarnitine excretion in the urine was high. Carnitine uptake in her cultured skin fibroblasts was less than 2% of normal at an extracellular carnitine concentration of 5 μmol/L.

Oral carnitine therapy (dose adjusted to maintain plasma carnitine in the low normal range) resulted in a rapid and marked improvement in cardiac function, with the echocardiogram and the electrocardiogram returning to normal, and increased muscle strength and growth. Now, at 16 years of age, she is entirely asymptomatic and in the 75th percentile for height and weight. She has shown an excellent academic performance at school.

2.2. Laboratory materials and methods

Cell cultures and DNA/RNA isolation. Lymphocytes were isolated and immortalized with Epstein Barr Virus and grown in 20% FBS/RPMI 1640/L-Glu at 5% CO_2 and 37 °C for patient 1 and both of his parents. DNA from cells was isolated by using a large scale salting-out method and total RNA was extracted by using RNA Stat (Tel-Test).

RT-PCR. The first strand OCTN2 cDNA was synthesized by RT-PCR using gene-specific anti-sense primers A-1932 (5′-GTGTCTGACTTGCTCCTGG-3′) and A-1305 (5′-AGTGGGCTATTTTGGGCTTT-3′). CDNA was synthesized using a superscript amplification RT_PCRsystem (Gibco BRL). The cDNA was amplified by PCR using, in addition to the above antisense primers, two sense primers S-45 (5′-GTCGTGCG CCCTATGTTAAG-3′), S-645 (5′-GTGTGAGGACG ACTGGAAGG-3′), and S-1112 (5′-GGACGATTTGA AGAGGCAGA-3′. The PCR products were purified by gel extraction (Qiagen) and used for either direct sequencing or subcloning.

Genomic PCR. Genomic DNA was isolated from cultured lymphocytes by using a large-scale salting out method. PCR was performed on exons 3 and 4 where the mutations were found on the cDNA level. The PCR products were then directly sequenced on an ABI Prism sequence analyzer.

Subcloning. The purified PCR products were subcloned into the PCR 2.1-TOPO plasmid vector provided in the TA cloning kit (Invitrogen). The cloned fragment was PCR amplified by using universal M13 vector primers. The PCR products were purified and analyzed by restriction enzymes, *Eae*III and *Nsp*I, to differentiate clones with the single bp deletion and 13 or 19 bp insertion. DNA sequences were run on ABI Prism sequence analyzer.

Carnitine uptake assay. The rates of carnitine uptake were measured in lymphoblast cell cultures grown in suspension. Cells (2×10^6) were resuspended in 2 mL serum free RPMI-1640 and incubated for 4 h with 2.5 Ci L-methyl-^3H carnitine at 37 °C and 5% CO_2 with various concentrations of unlabelled carnitine. After 4 h of incubation the cells were washed three times with PBS without Ca^{++} and Mg^{++}. Cells were then lysed in 2 mL of 0.5N NaOH and incubated at 37 °C for 30 min. The cell-bound radioactivity was measured by using scintillation counting. The cell protein was determined by the Lowry assay.

3. Results

Table 1 shows the results of the plasma membrane carnitine transport assay performed on cultured lymphocytes from patient 1 and his two parents. The rate of carnitine uptake was severely reduced in the patient's cultured lymphocytes to less than 2% of normal control cultured lymphocytes. Cultured lymphocytes from the patient's father and mother demonstrated intermediate rates of carnitine uptake with velocities of uptake reduced to 30% of normal control cultured lymphocytes. These are consistent with heterozygote carrier levels.

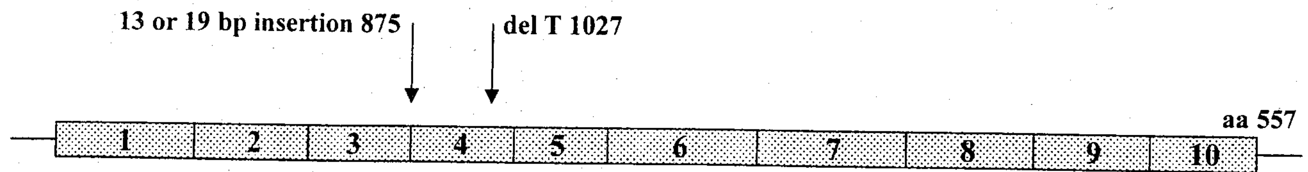
Screening of the coding exons and exon-intron boundaries of the OCTN2 gene by direct sequencing of genomic DNA revealed the presence of a homozygous frameshift mutation, 1027delT, in exon 4 of the OCTN2 gene in patient 1 (Fig. 1). As shown in the figure, this mutation is predicted to lead to a scrambled amino acid sequence at the site of the deletion, a premature stop codon at amino acid 295 in the OCTN2 protein and a loss of the C-terminal 262 amino acids of the normal 557

Table 1
Carnitine transport in transformed lymphocytes from patient 1, his parents and controls

	Carnitine uptake velocity (5 μM) (pmol carnitine uptake/min/mg protein) Mean ± SD
Proband	0.0032 ± 0.0006 (3)
Father	0.15 0.16
Mother	0.14 0.17
Controls	0.35 ± 0.11 (10)

n, Number of determinations.

A. OCTN2 coding region



B. Consequences of deletion and insertions

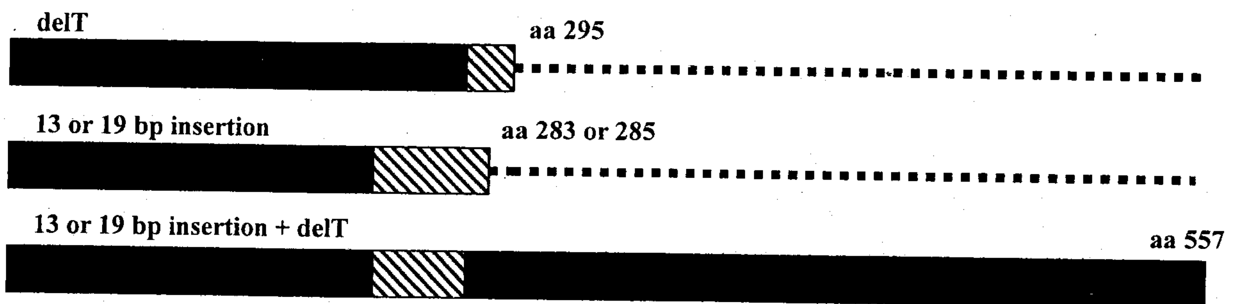


Fig. 1. Schematic representation of the protein encoded in the OCTN2 gene. (A) The protein with the 10 exons delineated and juxtaposed. The junction between exons 3 and 4 is the site of the 13 or 19 bp insertion and is marked by an arrow. The deletion of a T at position 1027 of the mRNA occurs at the 3′ end of exon 4 and is marked by a second arrow. (B) The consequences of the insertions and the single base pair deletion on the 557 amino acid OCTN2 protein. Del T at mRNA position 1027 results in a frame shift beginning at the site of the deletion and causing a scrambled amino acid sequence terminating with a stop signal at amino acid position 295. The normal sequence in this figure is represented by a black rectangle, the scrambled sequence by the hatched symbol and the missing amino acids by the broken line. Insertion of the 13 or 19 bp of intron 3 results in a scrambled sequence at the site of insertion and chain termination at amino acids 283 or 285. The presence of one of the insertions and the deletion results in a frame shift at the site of the insertion and a scrambled amino acid sequence at the exon 3–exon 4 border. A recovery of the proper frame occurs following the deletion and a full-length transcript is the result. The scrambled amino acids involve the critical ATP/GTP binding motif and disrupt membrane-spanning regions 5 and 6 (not shown in this figure). An inactive membrane transporter would be predicted.

amino acid sequence. Patient 1 was also homozygous for a silent polymorphism, 1028G > A, immediately following the frameshift mutation. Both of parents were heterozygous for the frameshift mutation, 1027delT in exon 4. The mother was heterozygous for the polymorphism, 1028G > A while the father was homozygous for the polymorphism.

Direct sequencing of cDNA prepared from patient 1's cultured lymphocytes confirmed the homozygous 1027delT and 1028G → A mutations in exon 4. However, it also revealed the presence of aberrant transcripts containing 13 or 19 nucleotide insertions between the junction of exons 3 and 4 (Fig. 1). The splice junctions of exons 3 and 4 were sequenced from genomic DNA and found to be normal.

As shown in Table 2, when the cDNA fragments from patient 1 were subcloned, the aberrant transcripts containing the 13/19 nucleotide insertions in cis to 1027delT mutation were predominant (89%) while only 11% of the transcripts contained the the 1027delT mutation alone. Clones of cDNA prepared from normal control cultured lymphocytes also showed the 13/19 nt insertion at a low frequency (6%). Analysis of cDNA subclones prepared from the patient's mother and father demonstrated presence of 13/19 nt insertion transcripts together with the 1027delT mutant allele in 44% of the subclones. In the mother, 11% of the clones contained the 13/19 nucleotide insert together with the the wild-type exon 4 sequence. In 18 clones examined, the father had none that contained the 13/19 nucleotide in combination with the 1028G → A polymorphism. In these parents, abnormal splicing was found in 93% of their 15 clones that contained the 1027delT mutation, compared with 6% abnormal splicing in clones lacking the mutation ($p < 0.0001$). Subclones of OCTN2 cDNA from a control fibroblast culture were also examined and showed the presence of the 13/19 bp insertion in 22% of clones, confirming that the intron 3 splicing variation was not an artifact of cultured lymphocytes.

Table 2
Distribution of mRNA transcripts from the transformed lymphocytes from patient 1, his parents and a control

	Del T (%)	13 or 19 bp insertion (%)	Del T + insertion (%)	Wild-type (%)
Patient	11		89	
Father	6		44	50
Mother		11	33	56
Control		5		95

4. Discussion

The results of these studies prove that the patient with primary carnitine deficiency that we first described in 1980 [4] has a deficiency of the plasma membrane carnitine carrier encoded by OCTN2. At the time the report was written, we assumed that carnitine deficiency, like many other inborn errors of metabolism, was due to an inborn error of enzymatic function. Such a defect made replacement therapy logical and more likely to be effective. The marked urinary excretion of replacement carnitine was interpreted as indicative of the effective uptake of carnitine by the GI tract. With the demonstration that a membrane transport defect was the underlying cause of other cases of systemic carnitine deficiency, this became the more likely cause of the disease in our patient as well. Proving it was important, since tissue concentrations of carnitine measured in this patient, both before and after therapy are almost unique and their applicability to other cases of carnitine transporter deficiency needed to be shown. With this study we have demonstrated that assumption to be correct.

There are few patients with plasma membrane carnitine transporter deficiency who have been treated for a prolonged period of time and who reflect the sustained efficacy of this treatment. These three patients represent more than 50 years of ongoing treatment and all have greatly improved cardiovascular and hepatic function. Patients 1 and 3 have apparently normal heart size and measurable cardiac function, whereas patient two, despite excellent functional performance, still has mild left ventricular dilatation.

Patients 2 and 3 never experienced any intellectual problems (despite a severe episode of hypoglycemia in patient three) and are now continuing to function at a normal or high cognitive level. Patient 1 suffered severe cerebral oxygen deprivation as well as hypoglycemia, and has evidence of cerebral atrophy on imaging and is handicapped, presumably as a result. Because so many other patients with systemic carnitine deficiency are cognitively normal, it is reasonable to believe that carnitine deficiency adversely affects cognition primarily in the context of predisposing to episodes of hypoketotic, hypoglycemic coma with secondary cerebral injury.

We have been informed of at least two other patients with 20 or more years of treatment experience who are also doing well (D. Valle and C. Sansariq, personal communication).

Despite this favorable long-term experience with carnitine replacement therapy, the pathobiology of the system gives some reason for caution. The mutated transport protein not only causes impaired renal tubular reabsorption, but also impaired cellular uptake. The low normal or slightly reduced plasma levels of carnitine achieved with most patients, may be insufficient to correct the intracellular deficiency completely and the consequences of this shortage over many years in heart and skeletal muscle is unpredictable. Both patients one and two achieved only partial correction of carnitine in muscle, even though they showed considerable clinical improvement [4,22]. Moreover, cardiac hypertrophy has been reported in some heterozygote carriers, a finding that may foreshadow future cardiac pathology in individuals with the carnitine transporter defect, particularly in the context of aging and additive cardiac risk factors such as hypertension and/or environmental factors which may act synergistically to lower efficiency of cardiac ATP production, despite continuing carnitine therapy [12].

The mutation analysis in patient 1 proved to be of unusual interest as well. Firstly the parents, despite the absence of known consanguinity, proved to be carriers of the same mutation. They come from the relatively large city of Durango in Northern Mexico and deny any knowledge of common geographic origin from a small community. Consequently it is difficult, absent more extensive mutation analysis in Spanish or Mexican patients with plasma membrane carnitine transporter deficiency, to know whether this founder mutation is of Amerindian or European origin.

The effect of the mutation of a single T at position 1027 in the coding sequence appeared to result in defective transporter function through two molecular mechanisms: directly in a small proportion of transcripts by acting as a nonsense mutation with premature protein termination; and indirectly, by inducing mis-splicing (Fig. 1). RT-PCR of the mRNA from patient 1's cultured lymphocytes revealed a high prevalence of a splicing variant which represents insertions of either 13 or 19 bp at the junction of exons 3 and 4, presumably by causing recognition of alternative splicing signals. Either insertion alone results in a frame shift and a shortened polypeptide of either 283 or 285 amino acids. In the presence of the deleted nucleotide, these deletions allow for the recovery of the correct reading frame after the deletion and completion of the polypeptide, but with a stretch of "scrambled" amino acids in the region of the critical ATP/GTP binding motif [1,2]. The proband always has the 13/19 bp insertion in cis with the 1027delT mutation. Therefore the proband will have almost full length cDNA with a scrambled sequence in between, and in addition to the prematurely truncated cDNA with the 1027delT mutation alone. The abundance of these mis-spliced sequences, assessed by sub-cloning individual messages is shown in Table 2.

Surprisingly, this mis-splicing occurs in normal transformed lymphocytes approximately 6% of the time. It occurred in 89% of the clones from the patient and in 44 and 33% of the deleted clones from his parents. In the father, 44% of all clones had both the Del T and the

insertion, whereas 6% had the Del T alone. In the mother all the Del T clones had an insertion. In the aggregate, these data demonstrate that this single base pair deletion exaggerates a tendency to mis-splice at this site.

A 19-bp insertion "tatggccatcaggttggag" between nucleotides G874 and G875 which results from a missplicing event just downstream of exon 3 of the OCTN2 gene (as this sequence contains part of the donor intronic sequence), has been previously reported as one of the disease causing mutations in a boy of Italian descent who was shown to be a compound heterozygote [7]. This 19-bp insertion creates a frameshift resulting in a premature stop codon, thus yielding a predicted truncated protein of 284 amino acids. This boy had a mean rate of carnitine uptake in his cultured skin fibroblasts that was 2% of controls at a carnitine concentration of 5 μmol/L.

The peculiar feature of coding sequence mutation leading to splicing and thus translation abnormalities at remote sites, has been described previously. Vockley et al. [23] described this for mutations in the gene encoding isovaleryl CoA dehydrogenase and we have recently described this in the carnitine mitochondrial translocase gene [24]. Stoffers et al. [25] have reported a coding sequence mutation in the insulin promoting factor 1 gene associated with early onset diabetes which yields alternately translated protein products. In this case alternative products of the mutation contribute to the pathogenesis of the disease because one of the resulting proteins appears to act in a dominant negative fashion. At this point the significance of missplicing secondary to coding sequence mutations of OCTN2 to the biology of carnitine metabolism and function is inapparent.

In summary, systemic carnitine deficiency due to mutations in the carnitine membrane transporter gene, appears to be a highly treatable disorder. The duration of the effective treatment appears to be at least 20 years and may be life-long. Efficacy of treatment longer than that reported here will have to be confirmed by follow-up studies, particularly given the documented cases of cardiomegaly in adult carriers. Because complete reversal of all pathology has not been accomplished, early diagnosis and treatment is important. Whether newborn screening by tandem mass spectrometry will ascertain presymptomatic cases is uncertain [26].

Acknowledgments

This work was supported by the Mental Retardation Research Program at UCLA and by NIH Grants HD 06576, HD 04612, and HD 36415 (UCLA), by The Heart and Stroke Foundation of Ontario Grant T 3746 (to I. Tein), and in part, by NIH Grants RR 00240 and DK 56268 (to C.A. Stanley).

References

[1] X. Wu, P.D. Prasad, F.H. Leibach, V. Ganapathy, 1998 cDNA sequence, transport function, and genomic organization of human OCTN2, a new member of the organic cation transporter family, Biochem. Biophys. Res. Commun. 246 (1998) 589–595.

[2] I. Tamai, R. Ohashi, J. Nezu, H. Yabuuchi, A. Oku, M. Shimane, Y. Sai, A. Tsuji, Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2, J. Biol. Chem. 273 (1998) 20378–20382.

[3] Y. Shoji, A. Koizumi, T. Kayo, T. Okata, T. Takahashi, K. Harada, G. Takada, Evidence for linkage of human primary carnitine deficiency with D5S436: a novel gene locus on chromosome 5q, Am. J. Hum. Genet. 63 (1998) 101–108.

[4] P.R. Chapoy, C. Angelini, W.J. Brown, J.E. Stiff, A. Shug, S.D. Cederbaum, Systemic carnitine deficiency a treatable inherited lipid-storage disease presenting as Reye's syndrome, New Engl. J. Med. 303 (1980) 1389–1394.

[5] M.E. Tripp, M.L. Katcher, H.A. Peters, E.F. Gilbert, S. Arya, R.J. Hodach, A.L. Shug, Systemic carnitine deficiency presenting as familial endocardial fibroelastosis: a treatable cardiomyopathy, New Engl. J. Med. 305 (1981) 385–390.

[6] L.J. Waber, D. Valle, C. Neill, S. DiMauro, A. Shug, Carnitine deficiency presenting as familial cardiomyopathy: a treatable defect in carnitine transport, J. Pediatr. 101 (1982) 700–705.

[7] A.M. Lamhonwah, I. Tein, Carnitine uptake defect: frameshift mutations in the human plasmalemmal carnitine transporter gene, Biochem. Biophys. Res. Commun. 252 (1998) 396–401.

[8] J. Nezu, I. Tamai, A. Oku, R. Ohashi, H. Yabuuchi, N. Hashimoto, H. Nikaido, Y. Sai, A. Koizumi, Y. Shoji, G. Takada, T. Matsuishi, N. 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.

[9] 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.

[10] N.L. Tang, V. Ganapathy, X. Wu, J. Hui, P. Seth, P.M. Yuen, R.J. Wanders, T.F. Fok, N.M. Hjelm, Mutations of OCTN2, an organic cation/carnitine transporter, lead to deficient cellular carnitine uptake in primary carnitine deficiency, Hum. Mol. Genet. 8 (1999) 655–660.

[11] 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.

[12] A. Koizumi, J.-I. 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.

[13] 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.

[14] E. Mayatepek, J. Nezu, I. Tamai, A. Oku, M. Katsura, M. Shimane, A. Tsuji, Two novel missense mutations of the OCTN2 gene (W283R and V446F) in a patient with primary systemic carnitine deficiency, Hum. Mutat. 15 (2000) 118.

[15] Y. Wang, M.A. Kelly, T.M. Cowan, N. Longo, A missense mutation in the OCTN2 gene associated with residual carnitine transport activity, Hum. Mutat. 15 (2000) 238–245.

[16] S.D. Cederbaum, N. Austed, J. Bernar, J. Edmond, Four-year treatment of systemic carnitine deficiency, New Engl. J. Med. 310 (1984) 1395–1396.

[17] A.G. Engel, C.J. Rebouche, D.M. Wilson, A.M. Glasgow, C.A. Romshe, R.P. Cruse, Primary systemic carnitine deficiency II. Renal handling of carnitine, Neurology 31 (1981) 819–825.

[18] W.R. Treem, C.A. Stanley, D.N. Finegold, D.E. Hale, P.M. Coates, Primary carnitine deficiency due to a failure of carnitine transport in kidney, muscle, and fibroblasts, New Engl. J. Med. 319 (1988) 1331–1336.

[19] I. Tein, Z.-W. Xie, The human plasmalemmal carnitine transporter defect is expressed in cultured lymphoblasts: a new non-invasive method for diagnosis, Clin. Chim. Acta 252 (1996) 201–204.

[20] R. Pons, R. Carrozzo, I. Tein, W.F. Walker, L.J. Addonizio, W. Rhead, A.F. Miranda, DiMauro, D.C. DeVivo, Deficient muscle carnitine transport in primary carnitine deficiency, Pediatr. Res. 42 (1997) 583–587.

[21] C.A. Stanley, S. DeLeeuw, P.M. Coates, C. Vianey-Liaud, P. Divry, J.-P. Bonnefont, J.-M. Saudubray, M. Haymond, F.K. Trefz, G.N. Brenningstal, Chronic cardiomyopathy and weakness or acute coma in children with a defect in carnitine uptake, Ann. Neurol. 30 (1991) 709–716.

[22] I. Tein, D.C. De Vivo, F. Bierman, P. Pulver, L.J. De Meirleir, I. Cvitanovic-Sojat, R.A. Pagon, E. Bertini, C. Dionisis-Vici, S. Servidai, Impaired skin fibroblast carnitine uptake in primary systemic carnitine deficiency manifested by childhood carnitine-responsive cardiomyopathy, Pediatr. Res. 28 (1990) 247–255.

[23] J. Vockley, P.K. Rogan, B.D. Anderson, J. Willard, R.S. Seelan, D.I. Smith, W. Liu, Exon skipping in IVD RNA processing in isovaleric acidemia caused by point mutations in the coding region of the IVD gene, Am. J. Hum. Genet. 66 (2000) 356–367.

[24] B.Y. Hsu, V. Iacobazzi, Z. Wang, H. Harvie, R.A. Chalmers, J.-M. Saudubray, F. Palmieri, A. Ganguly, C.A. Stanley, Aberrant mRNA splicing associated with coding region mutations in children with carnitine–acylcarnitine translocase deficiency, Mol. Genet. Metab. 74 (2001) 248–255.

[25] D.A. Stoffers, V. Stanojevic, J.F. Habener, Insulin promotor factor-1 gene mutation linked to early-onset type 2 diabetes mellitus directs expression of a dominant negative isoprotein, J. Clin. Invest. 102 (1998) 232–241.

[26] B. Wilcken, V. Wiley, K.G. Sim, K. Carpenter, Carnitine transporter defect diagnosed by newborn screening with electrospray mass spectrometry, J. Pediatr. 138 (2001) 581–584.