Novel OCTN2 Mutations: No Genotype–Phenotype Correlations: Early Carnitine Therapy Prevents Cardiomyopathy

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Primary systemic carnitine deficiency or carnitine uptake defect (OMIM 212140) is a potentially lethal, autosomal recessive disorder characterized by progressive infantile-onset cardiomyopathy, weakness, and recurrent hypoglycemic hypoketotic encephalopathy, which is highly responsive to L-carnitine therapy. Molecular analysis of the SLC22A5 (OCTN2) gene, encoding the high-affinity carnitine transporter, was done in 11 affected individuals by direct nucleotide sequencing of polymerase chain reaction products from all 10 exons. Carnitine uptake (at Km of 5 μM) in cultured skin fibroblasts ranged from 1% to 20% of normal controls. Eleven mutations (delF23, N32S, and one 11-bp duplication in exon 1; R169W in exon 3; a donor splice mutation [IVS3+1 G>A] in intron 3; frameshift mutations in exons 5 and 6; Y401X in exon 7; T440M, T468R and S470F in exon 8) are described. There was no correlation between residual uptake and severity of clinical presentation, suggesting that the wide phenotypic variability is likely related to exogenous stressors exacerbating carnitine deficiency. Most importantly, strict compliance with carnitine from birth appears to prevent the phenotype.

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KEY WORDS: carnitine transporter; fatty acid oxidation; organic cation transporter; cultured skin fibroblasts; hypertrophic cardiomyopathy

INTRODUCTION

Carnitine (β-hydroxy-γ-trimethylaminobutyric acid) is a small water-soluble quaternary amine that serves as an essential cofactor for the transport of long-chain fatty acids as acylcarnitine esters across the inner mitochondrial membrane and modulates the intramitochondrial acyl-CoA/CoA sulfhydryl ratio in mammalian cells [Bremer, 1983]. Carnitine concentrations in tissues are normally 20- to 50-fold higher than in serum [Stanley, 1982].
1987]. Thus, uptake into tissues occurs across a large concentration gradient maintained by an active transport system [Stanley, 1987]. Body carnitine stores are tightly regulated by the kidney [Engel et al., 1981]. Kinetic studies have demonstrated similar Km values of 2–6 μmol/L for carnitine transport in cultured skin fibroblasts, muscle, and heart [Tein et al., 1990; Stanley et al., 1991; Pons et al., 1997], suggesting that they share a common transporter, and much lower affinity Km values for human liver (500 μmol/L) and brain (>1,000 μmol/L) [Bieber, 1988]. The high-affinity carnitine transporter expressed in human skin fibroblasts is highly dependent upon a large sodium potential across the plasma membrane [Tein et al., 1996].

Primary systemic carnitine deficiency is due to a defect in the high-affinity carnitine transporter expressed in fibroblasts, muscle, heart, kidney, and lymphoblasts [Tein et al., 1990; Stanley et al., 1991; Tein and Xie, 1996; Pons et al., 1997]. This carnitine uptake defect (CUD) is potentially lethal, autosomal recessive disorder characterized by early childhood onset carnitine-responsive cardiomyopathy, with or without weakness, recurrent hypoglycemic hypoketotic coma, failure to thrive, and extremely low plasma and tissue carnitine concentrations (<5% control). There is microvesicular lipid accumulation in muscle and liver and a severe renal leak of carnitine [Tein et al., 1990]. Early diagnosis and treatment with l-carnitine supplementation is life-saving and reverses the pathology including the cardiac, motor, and hepatic dysfunction with cessation of episodic coma and a restoration of normal growth, although serum and tissue carnitine concentrations remain low due to a persisting renal leak of carnitine and the transporter defect.

A new member of the organic cation transporter family, OCTN2 cDNA, was cloned from a human placental trophoblast cell line [Wu et al., 1998]. OCTN2 cDNA (~3.2 kb) encodes a protein of 557 amino acids with 12 putative transmembrane domains. Wu et al. [1998] predicted that the amino and carboxy terminus face the cytoplasmic side of the membrane and that there was a long extracellular loop of 107 aa between the first two transmembrane domains, containing three potential N-glycosylation sites (Asn-57, -64, -91). There were also five potential protein kinase C-dependent (Ser-164, -225, -280, -322, -323) and one protein kinase A-dependent phosphorylation (Ser-402) site in putative intracellular loop domains. An ATP/GTP binding motif (GTEILGKS) [Walker et al., 1982; Saraste et al., 1990] was found in the intracellular loop between the fourth and fifth transmembrane domains (aa 218-225). The human gene SLC22A5 coding for OCTN2 has been sequenced in its entirety as part of the Human Genome Project and mapped to human chromosome 5q31 [Wu et al., 1998]. Tamai et al. [1998] cloned OCTN2 from a human kidney cDNA library and identified it as the physiologically important, high-affinity, sodium carnitine cotransporter in humans. We transfected a GFP full-length human OCTN2 cDNA into CUD lymphoblast cell lines and obtained functional restoration of l-[^3H]-carnitine uptake [Lamhonwah and Tein, 1999].

The first reported human mutations for the carnitine uptake defect were frameshifts resulting in truncated proteins [Lamhonwah and Tein, 1998]. Subsequently, other novel mutations have been reported including missense, nonsense, and splice site mutations as well as deletions and insertions [Burwinkel et al., 1999; Koizumi et al., 1999; Nezu et al., 1999; Tang et al., 1999; Vaz et al., 1999; Wang et al., 1999; Mayatepek et al., 2000; Wang et al., 2000]. In Japan, a 1% prevalence of individuals heterozygous for OCTN2 mutations has been documented, giving an estimated incidence of CUD of 1 in 40,000 births [Koizumi et al., 1999]. Echocardiographic in these carriers revealed a predisposition to late-onset benign cardiac hypertrophy [Koizumi et al., 1999]. Identification of human mutations is critical because of the progressive and lethal nature of this disorder and the high incidence of sudden unexpected infant deaths unless there is early diagnosis and prompt intervention [Tein et al., 1990; Stanley et al., 1991]. Given the wide phenotype variability in this disease, correlations between genotype and phenotype may help predict the clinical course in affected individuals. In this study, we report additional new mutations in four pedigrees and five unrelated individuals with CUD and examine the correlations between these mutations and their respective clinical and biochemical phenotypes. In addition, we identify common mutations as well as a potential “hot spot” region in exon 5 and consider their potential impact on structure/function relationships.

**MATERIALS AND METHODS**

**Clinical and Biochemical Studies**

All studies on the patients were performed with prior informed parental consent and with the approval of the institutional review boards of the various universities and hospitals. Total and free carnitine concentrations in plasma, urine, and muscle were measured as described by McGarry and Foster [1976] for case 1. Dicarboxylic acids were identified in the urine as the trimethylsilyl derivatives by gas–liquid chromatography and were confirmed by gas chromatography–mass spectroscopy. Urinary fractional excretion of carnitine was calculated on the basis of the ratio of the clearance of carnitine to the clearance of creatinine.

**Cell Culture**

The cell lines were cultured skin fibroblasts and lymphoblasts obtained from normal human controls, from patients with the CUD, and from parents of the patients. Fibroblasts were grown in 100-mm² dishes containing alpha-MEM + 10% fetal calf serum (FCS) and lymphoblasts were grown in 25-cm² Falcon tissue culture flasks in RPMI 1640 with glutamine and 15% FCS in the presence of 5% CO₂ at 37°C.

**Carnitine Uptake Studies**

Carnitine uptake studies in cultured skin fibroblasts were performed as outlined in Tein et al. [1990] for cases 1 to 8 and their respective parents, where available,
in our laboratory (controls: $K_m = 4.8 \mu M$; $V_{max} = 1.82$ pmol/min/mg protein) and by a modification of the method of Treem et al. [1988] for cases 9 to 11 in Dr. C. Vianey-Saban's laboratory. Carnitine uptake studies in cultured lymphoblasts were performed in case 3 according to Tein and Xie [1996] in our laboratory (controls: $K_m = 4.4 \mu M$; $V_{max} = 2.06$ pmol/min/mg protein).

L-[3H]-carnitine (0.1 Ci/ml; Amersham BioSciences Inc., Quebec, Canada) was used in the carnitine uptake experiment at 5 $\mu$mol/L carnitine ($K_m$ value for the high-affinity carnitine transporter OCTN2) and at 10 mM carnitine for nonspecific uptake. Control values were established from carnitine uptake studies in 36 normal control cell lines. Full kinetic studies of carnitine uptake at varying concentrations of carnitine were done to determine $K_m$ and $V_{max}$ values in affected individuals, heterozygotes and controls [Tein et al., 1990]. Patient cell lines were subsequently entered into the study for mutational analysis after confirmation of markedly impaired skin fibroblast carnitine uptake ($<20\%$ of controls) at the $K_m$ concentration for carnitine uptake (5 $\mu$mol/L carnitine).

RNA Purification
Total RNA was harvested using a Qiagen RNeasy total RNA isolation kit or using Trizol Reagent (Gibco BRL, Grand Island, New York).

Reverse Transcriptase Polymerase Chain Reaction Analysis of mRNA
The cDNA was synthesized as described previously [Lamhonwah and Tein, 1998].

Subcloning and Nucleotide Sequencing of Reverse Transcriptase Polymerase Chain Reaction Products
The cDNA amplicons were prepared for direct sequencing or for subcloning as described previously [Lamhonwah and Tein, 1998].

Genomic DNA Isolation
For Cases 1 to 8 and their respective parents, genomic DNA was purified from cultured skin fibroblasts and lymphoblasts using the QIAamp Blood Kit (Qiagen Inc., Valencia, CA). For Cases 9, 10 and 11, total DNA was purified from the buffy coat obtained from blood samples from the three siblings and from both of their parents.

Genomic Amplification of the 10 Exons of the Human SLC22A5 Gene Encoding the High-Affinity Carnitine Transporter OCTN2 by Polymerase Chain Reaction
Specific oligonucleotide sequences flanking each of the 10 exons were designed from the nucleotide sequences under Genbank Accession number AC004628 and AF057164 (contact authors for sequence of amplimers, annealing temperature, size of polymerase chain reaction [PCR] product, and final MgCl$_2$ concentration used). Exon 1 was missing in the sequence from Genbank Accession number AC004628 and thus, in an attempt to amplify exon 1, synthetic oligonucleotides hcnt1-3A from AC004628 sequence and oligo S-21 from AF057164 were used to amplify genomic DNA from normal control individuals. For each of the 10 exons, genomic DNA (300 ng) from each sample was amplified in a 50-$\mu$L reaction volume containing 20 mM Tris HCl pH 8.4, 50 mM KCl, 0.2 mM dNTPs, 2.5 U of Taq polymerase (Invitrogen Canada Inc., Burlington, Ontario, Canada), 10% DMSO, and MgCl$_2$ in specified concentrations. A 15-$\mu$L aliquot of the amplified genomic fragments was analyzed using a 1% agarose/1X TBE buffer, and each band was excised from the agarose gel. The PCR products were purified from the agarose bands using the GeneClean II kit (Bioloi, Vista, CA) and were used subsequently for direct sequencing analysis.

Direct Nucleotide Sequencing of PCR Products
Direct nucleotide sequencing of the PCR products was done using the ThermoSequenase Cycle Sequencing kit (Amersham) and $^{35}$S-dATP and $^{35}$S-dCTP (Amersham). The sequencing reactions were done in a Perkin Elmer Thermal Cycler and analyzed on Sequagel-6 (National Diagnostics, Atlanta, GA). The two internal primers used for exon 1 sequencing were S-330 (5'CTGTCC-TCCGTTTCTAGTAGC-3') and A6-401(5'GCTGC- TCAATTGCAGCGGTCTC-3'). The dried sequencing gel was exposed to Biomax X-ray film (Kodak, Rochester, NY) from 6 hr to overnight for autoradiography.

RESULTS
Clinical Data
The hallmark features of the 11 index cases in our series, diagnosed to have CUD based upon confirmatory fibroblast carnitine uptake studies, are summarized in Table I (clinical features) and Table II (biochemical features). These tables illustrate the phenotypic variations in age of onset or detection, presenting feature(s), associated biochemical abnormalities, and the clinical outcome. Selected case histories are presented in detail to highlight the phenotypic variation within families and the critical importance of early intervention with carnitine therapy and therapeutic compliance. Cases 1 and 2 have been previously reported [Tein et al., 1990].

Case 3 (L32). A 2$\frac{1}{2}$-yr-old girl of Croatian origin presented at 6 mo of age with a Reye-like syndrome with hypoketotic hypoglycemic encephalopathy, seizures, and elevated liver transaminases up to 1,000 U/L and ammonia up to 222 $\mu$mol/L [Maradin et al., 2000]. Serum glucose was reduced to 1.1 mmol/L. At this time she had mild hypertrophic cardiomyopathy involving primarily the left ventricle on echocardiography, which progressed to moderate over the next 2 mo. Initial electrocardiogram and chest radiograph at 6 mo were normal. She was also noted to be hypotonic and weak with gross motor delay and to have failure to thrive. Her myopathy was confirmed by electromyogram at 10 mo. Serum total carnitine before therapy was 0.78 $\mu$mol/L and free carnitine was 0.72 $\mu$mol/L. Urine organic acids demonstrated increased excretion of 3-methylglutaconic acid.
<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Ethnicity</th>
<th>Consanguinity</th>
<th>Affected sibs</th>
<th>Age at presentation (mon)</th>
<th>Presenting feature</th>
<th>Cardiomyopathy</th>
<th>Myopathy or motor delay</th>
<th>Hypoglycemic encephalopathy</th>
<th>Hypotonia</th>
<th>Failure to thrive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (F11)</td>
<td>F</td>
<td>East Indian/Irish</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>Cardiac\textsuperscript{a}</td>
<td>+ (Dil)\textsuperscript{a}</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. (F12)</td>
<td>F</td>
<td>Croatian</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>Hypotonia</td>
<td>+ (Hyp)\textsuperscript{d}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3. (L32)</td>
<td>F</td>
<td>Croatian</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>Coma, cardiac,\textsuperscript{a} anemia, motor delay,\textsuperscript{c} failure to thrive</td>
<td>+ (Hyp)\textsuperscript{c}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4. (E1136)</td>
<td>F</td>
<td>Caucasian</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>Coma,\textsuperscript{b} hypotegaly</td>
<td>+ (Hyp)\textsuperscript{b}</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5. (E1137)</td>
<td>M</td>
<td>Caucasian</td>
<td>-</td>
<td>+</td>
<td>60</td>
<td>Coma\textsuperscript{c}</td>
<td>+ (Dil)\textsuperscript{c}</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6. (E1245)</td>
<td>F</td>
<td>Asian</td>
<td>+</td>
<td>-</td>
<td>2</td>
<td>Coma, hypotonia, hepatomegaly</td>
<td>LVH\textsuperscript{f} on EKG</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7. (E1936)</td>
<td>M</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8. (E1808)</td>
<td>M</td>
<td>Asian</td>
<td>+</td>
<td>-</td>
<td>27</td>
<td>Coma\textsuperscript{d}</td>
<td>+ (Hyp)\textsuperscript{d}</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9. (P1)</td>
<td>M</td>
<td>Turkish</td>
<td>-</td>
<td>+</td>
<td>36</td>
<td>Cardiac,\textsuperscript{a} anemia, hypotonia, motor delay,\textsuperscript{e} hepatomegaly</td>
<td>+ (Hyp)\textsuperscript{e}</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10. (P2)</td>
<td>F</td>
<td>Turkish</td>
<td>-</td>
<td>+</td>
<td>8 days Carnitine tx\textsuperscript{h} at birth</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11. (P3)</td>
<td>M</td>
<td>Turkish</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Cardiac, cardiomyopathy.
\textsuperscript{b}Coma, hypoglycemic encephalopathy.
\textsuperscript{c}Dil, dilatative cardiomyopathy.
\textsuperscript{d}Hyp, hypertrophic cardiomyopathy.
\textsuperscript{e}Motor delay, myopathy and/or motor delay.
\textsuperscript{f}LVH, left ventricular hypertrophy.
\textsuperscript{g}No apparent hypoglycemia.
\textsuperscript{h}tx, therapy.
## TABLE II. Biochemical and Molecular Features and Response to l-Carnitine in Individuals with Carnitine Transporter Defect

<table>
<thead>
<tr>
<th>Case</th>
<th>Affected exon mutation</th>
<th>Plasma carnitine (total/free) μM</th>
<th>Plasma carnitine (total/free) μM</th>
<th>Urine organic acids</th>
<th>% of control Cn uptake at 5 μM Cn in fibroblasts</th>
<th>Beneficial response to Cn therapy</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pretherapy</td>
<td>Posttherapy</td>
<td>Mother</td>
<td>Father</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 1. (F11) | Ex 1: g.67-69del TTC  
Intron 3:  
g.14944G > A  
Intronic mutations | 19/15 | 25–60/17–33 | 36/28 | 61/55 | N | 2.0 | C/F/M | Alive at 19.5 yr; asymptomatic, bright; normal LV function; mild LV dilatation |
| 2. (F12) | Ex 5: g.17081 del C  
homozygous | 0 | 5 | N | 1.1 | C/E/M | Alive at 15.7 yr, asymptomatic, bright |
| 3. (L32) | Ex 8: g.22521 C > T  
homozygous | 0.78/0.72 | 10–36/5–28 | 33 | 31 | 3-MG | 2.8 (0.5<sup>a</sup>) | C/E/F/H/M | Alive at 2.5 yr, borderline hypotonia |
| 4. (E1136) | Ex 3: g.14196 C > T  
Ex 8: g.22521 C > T  
homozygous | 10.8/10.4 | 9.2/3.2 | N | 1.1 | C/E/M | Alive at 2.5 yr, asymptomatic, bright |
| 5. (E1137) | Ex 7: g.20876-7 insA  
homozygous | 10.8/10.4 | 9.2/3.2 | N | 1.1 | C/E/M | Alive at 2.5 yr, asymptomatic, bright |
| 6. (E1245) | Ex 1: g.95A > G  
homozygous | 10.8/10.4 | 9.2/3.2 | N | 1.1 | C/E/M | Alive at 2.5 yr, asymptomatic, bright |
| 7. (E1806) | Ex 1: g.265 dup 11  
Ex 8: g.22605 C > G | Very low | Very low | N | 1.1 | C/E/M | Alive at 2.5 yr, asymptomatic, bright |
| 8. (E1808) | Ex 8: g.22611 C > T  
homozygous | 15.4/2.7 | 15.4/2.7 | N | 1.1 | C/E/M | Alive at 2.5 yr, asymptomatic, bright |
| 9. (P1) | Ex 6: g.19015 del A  
Ex 8: g.22521 C > T | 2/1 | 22–76/14–55 | 40/34 | 40/34 | DCA | 16<sup>b</sup> | C/F | Alive at 10 yr, muscle cramps, mild delay |
| 10. (P2) | Same as P1 | 4/0 | 16–50/13–40 | 40/34 | 40/34 | DCA, MC | 16<sup>b</sup> | C/F | Alive at 4 yr; well |
| 11. (P3) | Same as P1 | 2/0 | 4/2 at 12 hr postdose | 40/34 | 40/34 | MC | 16<sup>b</sup> | Cn tx at birth | Alive at 3 yr; well |

C, cardiomyopathy; Cn, carnitine; D, dilatative cardiomyopathy; DCA, dicarboxylic aciduria; E, hypoglycemic encephalopathy; EKG, electrocardiogram; Ex, exon; F, failure to thrive; H, hypotonia; L, liver enlargement; LV, left ventricular; LVH, left ventricular hypertrophy; M, myopathy/motor delay; MC, methylcitrate excretion; 3-MG, 3-methylglutaconic aciduria; N, normal; tx, therapy.

<sup>a</sup>Studies done in cultured lymphoblasts.

<sup>b</sup>Percent uptake values obtained from Dr. C. Vianey-Saban’s laboratory.
and 3-methylglutaric acids and occasional mild dicarboxylic aciduria. She had decreased renal reabsorption of carnitine prior to therapy at a time of extremely low serum carnitine concentrations. She also had a microcytic anemia (hemoglobin of 8.6 g/dL). Diagnosis was confirmed on the basis of impaired cultured lymphoblast carnitine uptake. After diagnosis at 8 mo of age, she was treated with 150 mg/kg/d of carnitine during the first year of therapy and with 100 mg/kg/d thereafter. The hypertrophic cardiomyopathy resolved by 17 mo of age. Similarly, her hypotonia, weakness, and gross motor delay resolved. She suffered no further episodes of coma. Her weight increased from below the 3rd centile at 6 mo to the 10th centile by 21/2 yr. Serum total carnitine concentrations varied between 10 and 36 μmol/L with free carnitines of 5–28 μmol/L. There was a marked reduction in urinary 3-methylglutaconic acid excretion. Hemoglobin increased to only 10.5 g/dL despite iron therapy; however she suffered from intercurrent salmonellosis and enterobiasis. Serum carnitine concentrations in her parents were normal as in her asymptomatic siblings at 29–36 μmol/L. At 21/2 yr, she has borderline hypotonia.

**Case 9 (P1).** This 10-yr-old boy, born to nonconsanguineous Turkish parents, has two affected siblings (cases 10 and 11). He presented at 5 yr of age with hypotonia, hypertrophic cardiomyopathy, and myopathy with an elevated creatine kinase level of 784 U/L. He also had hepatomegaly with a mild elevation in transaminases and a hypochromic, microcytic iron deficiency anemia (hemoglobin of 8.3 g/dL). His serum total and free carnitine, respectively, were markedly reduced at 2 and 1 μmol/L and he had decreased renal reabsorption of carnitine. Urinary organic acids revealed increased methylcitrate. On diagnosis at 31/2 yr, he was treated with 200 mg/kg/d of carnitine, increased to 300 mg/kg/d at 9 yr and 400 mg/kg/d at 91/2 yr, with good compliance. His serum total and free carnitines, respectively, varied from 22 and 14 μmol/L before the morning dose to 76 and 55 μmol/L 2 hr postdose. His myopathy improved, although he had nocturnal leg cramps a day after a day of sports. His cardiomyopathy was improved from 71/2 yr of age. He never suffered from hypoglycemic coma. His mother has a normal serum carnitine level. At 10 yr of age, his examination is normal. He has muscle cramps every 3 days and is 1 year behind in school.

**Case 10 (P2).** This 4-yr-old girl is the sister of case 9. She presented at 8 days of age with a mild hypertrophic cardiomyopathy, bradycardia, and atrial extrasystoles. Her serum total and free carnitine, respectively, were markedly reduced at 4 and 0 μmol/L and she demonstrated a decreased renal reabsorption of carnitine. Her urine organic acids revealed a slight dicarboxylic aciduria with a mild increase in methylcitrate excretion. She subsequently demonstrated hypotonia with gross motor delay, only walking at 18 mo, and suffered from a microcytic, hypochromic, iron deficiency anemia. She also had failure to thrive between 6 to 20 mo with weight −2.5 SD below normal, but normal height. There have been no episodes of hypoglycemic encephalopathy. She was started on 100 mg/kg/d of carnitine at 9 d of age, increased to 400 mg/kg/d at 15 mo of age, although compliance has been variable. Her serum total and free carnitine, respectively, varied from 16 and 13 μmol/L before the morning dose to 50 μmol/L and 40 μmol/L 4 hr postdose. After therapy, there was an improvement in her cardiomyopathy, although it remains moderate. There has been an improvement in her weight centile to −1 SD at 4 yr, particularly when there has been good compliance with carnitine. She is asymptomatic at 4 yr of age, attending kindergarten.

**Case 11 (P3).** This 3-yr-old boy is the brother of cases 9 and 10. His serum total and free carnitine, respectively, at birth were 2 and 0 μmol/L. Of note, his mother’s serum total and free carnitine, respectively, 1 day after delivery were reduced at 18 and 14 μmol/L. This newborn’s urine organic acids revealed a slightly increased methylcitrate excretion. Given his low serum carnitines and his previously diagnosed siblings, he was treated with carnitine from birth at an initial dose of 200 mg/kg/d, which was increased to 400 mg/kg/d at 7 mo of age with good therapeutic compliance. When his serum carnitine was tested 12 hours after his carnitine dose, his serum total carnitine was <4 μmol/L and the free carnitine was <2 μmol/L. He has remained asymptomatic with no evidence of myopathy, hypotonia, epidosic hypoglycemia, gross motor delay, or failure to thrive. He has had no signs of cardiomyopathy but has an interventricular septum at the upper limit of control values. He has a microcytic, hypochromic, iron deficiency anemia but is well at 3 yr of age.

**Molecular Analysis of the SLC22A5 (OCTN2) Gene in Individuals with CUD**

Genomic samples from five concomitant normal controls, 11 individuals with the carnitine uptake defect, and eight carriers from four confirmed pedigrees were amplified for all 10 exons of the SLC22A5 (OCTN2) gene. For five affected individuals, cells from the parents were not available to confirm the allelic inheritance of the mutations found. For each of the 10 exons of the OCTN2 gene, the size of the genomic amplicons expected with each pair of amplimers varied from approximately 260 bp to 2.7 kb. We excised the PCR products and performed direct nucleotide analysis for each of the 10 exons. This enabled us to rapidly identify disease-causing mutations and silent polymorphisms. All 10 exons were sequenced in entirety for each of the affected individuals, carriers, and controls. Direct nucleotide analysis of each of the 10 exons was done with the same amplimers used for genomic PCR amplification. In the case of exon 1 (approximately 2.7-kb-long PCR product), two internal primers—S-330 and A6-401—were used in the sequencing reactions such that the intronic sequences flanking exon 1 were covered. Exons 7, 8, and 9 were subsequently amplified in one stretch of approximately 3 kb and the PCR products were directly sequenced with the primers flanking each of the respective exons 7, 8, and 9. The results of our sequencing strategy in the analysis of the mutations in these 11 individuals with CUD, and in their respective heterozygote parents compared to normal controls are shown in Figures 1 and 2. Each figure displays the
Novel OCTN2 Mutations

autoradiographs of the partial sequence around the site of the mutation as well as the predicted impact of the mutation(s) in the affected individual. The genomic nucleotide numbering is done according to Genbank accession number AB016625 sequence [Nezu et al., 1999], with the exception that the first 221 nucleotides, upstream of the open reading frame, were subtracted each time as recommended by the nomenclature system of Antonarakis [1998]. Thus, nucleotide 1 is A of the initiator ATG methionine of the SLC22A5 gene encoding the human OCTN2.

In the following section, we report the mutations in the SLC22A5 (OCTN2) gene in our series of 11 individuals with CUD. Overall, the carnitine uptake at 5 μmol/L of carnitine (Km for the plasmalemmal high-affinity carnitine transporter) in the affected individuals ranges from 1% to 20% of controls. Both mutant alleles (allele 1 and allele 2) are described as well as the predicted impact of each mutation. None of these mutations were present in the DNA of 26 normal control alleles that were screened.

Case 1 (F11, Figure 1A & B) is of East Indian/Irish origin and is heterozygous for two different mutations. Allele 1 is the maternal (East Indian) mutant allele, which harbors a 3-bp deletion in exon 1 at nucleotide g.67-69 of the OCTN2 gene. This mutation was originally found in reverse transcriptase–PCR subclones of RNA samples from both the mother and index case. This 3-bp deletion results in the deletion of Phe23 in the putative first transmembrane domain and may change the conformation or topology of a functional OCTN2 protein. The second mutation in case F11, inherited from her Irish father and designated as allele 2, is a single nucleotide substitution (g.14344G > A) located at the splice donor site at the 5' end of intron 3. This mutation, described as IVS3+1G > A, would have a splicing effect. The biochemical consequence of compound heterozygosity for these two mutations is a reduction in carnitine uptake to 2% of control values in fibroblasts. In previous work, we have shown that both parents have normal Km values but a reduction in Vmax (40% of controls in mother; 22% in father) [Tein et al., 1990], suggesting that this mutation does not interfere with carnitine binding.

Case 3 (L32, Fig. 1D) is a girl of Croatian origin who is homozygous for a single nucleotide substitution in exon 8 at g.22521C > T, which results in a missense mutation, Thr440Met. This replacement of a highly conserved, polar, uncharged threonine by a nonpolar, hydrophobic methionine residue occurs in transmembrane domain X and may interfere with the topology or conformation of the transporter in the membrane. It is interesting to note that the affected child has 2.8% of control carnitine uptake in her cultured skin fibroblasts and only 0.5% of control carnitine uptake in her cultured lymphoblasts at 5 μmol/L carnitine. Although her clinical and biochemical presentation and response to l-carnitine therapy have been entirely consistent with the high-affinity carnitine transporter defect, this individual is unique given the novel occurrence of 3-methylglutaconic aciduria [Maradini et al., 2000], which may suggest an additional metabolic defect, yet to be defined. Both parents (L37 and L36) are heterozygous for the Thr440Met mutation. On kinetic studies in cultured lymphoblasts from the parents, the Km values for carnitine uptake are within the normal range but the Vmax is reduced to 27% of controls in the mother and 12% in the father, suggesting other modifying genetic factors.

Case 4 (E1136, Fig. 1E and F) is heterozygous for the g.22521C > T mutation in exon 8 (T440M) and the g.14196C > T mutation in exon 3 (Arg169Trp). The carnitine uptake in cultured skin fibroblasts is 20.5% of normal control values, in contrast to case 3, who have shown to be homozygous for the Thr440Met and to have negligible carnitine uptake (2.8% of controls). This may suggest some genetic complementation between these two mutations. Alternatively, given the unexpected 3-methylglutaconic aciduria in case 3, there may be additional modifying genetic factors influencing carnitine transport in case 3. The R169W missense mutation replaces a basic, positively charged arginine with a nonpolar, hydrophobic tryptophan residue in the cytoplasmic loop between transmembrane domains II and III. This arginine 169 occurs in the “sugar transporter sequence signature” (aa 160-176) [Nezu et al., 1999] and is highly conserved in the transporter superfamily to which OCTN2 belongs. The corresponding sequence position is occupied by arginine not only in the mammalian organic cation and anion transporters, but even in mammalian facilitative sugar permeases (e.g., mammalian glucose transporter GLUT 1) and bacterial proton-driven sugar transporters (e.g., bacterial xylose and arabinose transporters) [Burwinkel et al., 1999]. Burwinkel et al. [1999] have recently reported an affected individual who was heterozygous for Arg169Gln and Arg282ter.

Case 5 (E1137, Figure 1G) is homozygous for a 1-bp insertion at g.20876-20877insA in exon 7 of the OCTN2 gene, resulting into a premature stop codon, Tyr 401 ter, and a truncated protein that is shortened by 156 aa. This highly conserved tyrosine is the first residue in transmembrane domain IX and may thus serve as the 3' anchor of the preceding intracellular loop. The biochemical consequence of this mutation is a reduction in...
Fig. 1. Sequence of the OCTN2 gene around the site of mutations in six patients with the carnitine uptake defect and the impact of the mutations. Genomic DNA was amplified by PCR and sequenced. A and B: The 3-bp deletion in exon 1 (delF23) and the g. 14344 G > A at the donor splice sequence of intron 3 in case F11 (missplicing) respectively. C: Case F12 is homozygous for the g.17081delC in exon 5 (frameshift). D: Case L32 is homozygous for the g. 22521 C > T in exon 8 (T440M). E and F: Case E1136 is heterozygous for the g. 14196 C > T mutation in exon 3(R169W) and the g.22521C > T in exon 8 (T440M). G: Case E1137 is homozygous for the g. 20876-20877insA in exon 7 (Y401X). H: Case E1245 is homozygous for the g. 95A > G in exon 1 (N32S).
fibroblast carnitine uptake to 4.8% of normal controls. This Y401X mutation has been previously reported in a Caucasian heterozygous for this mutation and expression of this mutant cDNA in CHO cells was shown to result in negligible carnitine uptake compared to wild-type cDNA [Wang et al., 1999].

Case 6 (E1245, Figure 1H) is of Asian origin and is homozygous for the single nucleotide substitution of g.95 A > G in exon 1, resulting in Asn32Ser. This individual has 20.6% carnitine uptake in cultured skin fibroblasts at 5 μmol/L carnitine. This asparagine 32 residue is highly conserved and occurs in the first

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transmembrane domain. Thus, a mutation in this region may interfere with the topology or conformation of the transporter. The somewhat higher residual carnitine uptake may reflect the fact that the polar uncharged asparagine residue is replaced by the polar uncharged serine residue. During the preparation of this article, we became aware of a recent report describing two Danish individuals (both from the Faroe Islands) who were homozygous for this Asn32Ser mutation [Christensen et al., 2000]. As this N32S mutation has been found both in Asiatic and Danish families, it may be a recurrent mutation that has arisen in diverse genetic backgrounds or else a very ancient founder mutation.

Case 7 (E1936, Fig. 2A–C) is heterozygous for a mutation in exon 1 (an insertion of 11 bp that is a tandem duplication of nucleotide g.254-264 of the OCTN2 gene, namely, g.265ins/dup GGCTGCCCACC). This 11-bp insertion could result from an unknown mechanism during DNA recombination. However, this 11-bp mutation in exon 1 results in a frameshift leading to an Ile substitution by Gly at position 89 and a predicted truncated OCTN2 chimeric protein of 132 aa. Furthermore, a silent polymorphism at nucleotide g.246 C > T (no change in Arg82) is observed. Nucleotide sequencing of several subclones of the exon 1 PCR product from this case revealed that the mutant allele with the g.265ins/dup11 has the g.246T haplotype. The second mutation in this case is a single nucleotide substitution at g.22605 C > G in exon 8 of the OCTN2 gene, resulting in the substitution of a polar, uncharged threonine at codon 468 by a positively charged arginine in transmembrane domain XI. This change could interfere with the conformation of the transporter protein in the membrane. The biochemical consequence of this compound heterozygosity is a marked reduction in carnitine uptake to 5.9% of controls. Most of this residual activity is likely attributable to the protein encoded by the mutant allele with the g.22605 C > G mutation, as the 11-bp insertion truncates the protein by 425 aa and leads to a loss of transmembrane domains II to XII. Koizumi et al. [1999] have identified a mutation, Ser467Cys, just upstream of our T468R mutation, in four Japanese carriers. In expression studies in HEK 293 cells, they showed that the S467C mutant cDNA reduced L-carnitine uptake to 11% of wild-type cDNA.

Case 8 (E 1808, Fig. 2D) is homozygous for a single nucleotide substitution at g.22611 C > T in exon 8 of the OCTN2 gene, resulting in a missense Ser470Phe mutation. Substitution of this polar uncharged serine residue at codon 470, which is a highly conserved amino acid residue in transmembrane domain XI, by a nonpolar, hydrophobic phenylalanine residue may interfere with the topology of the transporter in the membrane. The biochemical consequence of this mutation was a marked reduction in fibroblast carnitine uptake to 7.1% of normal controls.

Cases 9, 10, and 11 (P1, P2, P3, Fig. 2E and F) are three siblings of Turkish origin who are heterozygous for a 1-bp deletion (g.19015delA) in exon 6 of the OCTN2 gene. This 1-bp deletion is inherited from the mother and the mutation results in a frameshift with Thr337Pro leading to a predicted truncated protein of 347 aa. The second mutation is a single nucleotide substitution of g.22521 C > T in exon 8 and is inherited from the father as shown by the pedigree. The paternal mutation results in a Thr440M substitution, which has also been found in Case 3 who is homozygous for this mutation as well as in case 4, who is similarly a compound heterozygote. It is interesting to note that the T440M mutation results in a more significant reduction in carnitine uptake in the homozygous state (2.8% residual uptake) compared to the compound heterozygous state (16–20%) suggesting, as one possibility, that there may be partial genetic complementation in cases 4 (T440M/R169W) and cases 9–11 (T440M/frameshift exon 6).

DISCUSSION

This study and previous studies indicate that the carnitine uptake defect (CUD) most commonly presents with signs of progressive cardiomyopathy in late infancy or early childhood usually between 1 and 7 yr of age (median, 3 yr) [Tein et al., 1990; Garavaglia et al., 1991; Stanley et al., 1991]. Affected individuals are generally normal at birth and may appear healthy for several years until they develop overt signs of cardiomyopathy and congestive heart failure, which may be rapidly fatal unless they are diagnosed and treated with carnitine. Alternatively, individuals with the CUD may present with episodic hypoglycemic, hypoketotic coma, which tends to occur at a younger age, between 1 month to a maximum of 5 years in this series, and with an estimated median of approximately 1.5 yr [Stanley et al., 1991]. All demonstrate a beneficial response to carnitine therapy, provided there is good compliance. Both clinical presentations may occur in one family. Stanley et al. [1991] reported a family in which one boy presented with hypoglycemia in early infancy, whereas his brother presented with cardiomyopathy at 7 yr of age. Garavaglia et al. [1991] also reported a family in which one boy presented with hypoglycemia and myopathy but no cardiomyopathy, whereas his brother died at an early age with autopsy findings of a dilated cardiomyopathy and low cardiac carnitine concentrations.

Given the variation in clinical phenotype within the same family, it appears that other exogenous factors relating to fasting stress, infection, a carnitine-deficient diet, or use of drugs that induce carnitine deficiency such as pivalic acid [Holme et al., 1989] or valproic acid [Tein et al., 1993], may provoke an acute episode of illness before the cardiomyopathy is fully developed [Stanley et al., 1991]. This is highlighted by the sudden neonatal death on day 5 of life of a child with the carnitine transporter defect [Rinaldo et al., 1997]. This occurred during fasting stress caused by poor breast-feeding (no formula supplements) and possibly the vegetarian diet of the mother. Similarly, in the current study we report an Asian child, homozygous for the N32S mutation, who presented with hypoglycemic coma at 2 mo of age in the context of a diarrheal illness, who was subsequently treated with carnitine and who is currently 64 years of age with no overt cardiomyopathy. Furthermore, a child from the Faroe Islands [Christensen et al., 2000] with the same N32S/N32S genotype died unexpectedly at 14 mo of age after 1 day of illness with a fatty liver in the
context of pivampicillin therapy, which likely exacerbated the severe carnitine deficiency. Pivalolglycine was found in the urine. Postmortem blood carnitine concentration was very low (0.7 μmol/L). The authors suggested that pivampicillin contributed to sudden infant death in their case.

The clinical profiles in our case 2, who is homozygous for a single nucleotide deletion of g.17081C in exon 5 leading to a frameshift at R282D and predicted truncated protein of 294 aa and those for the three cases that were homozygous for the R282X mutation [Burwinkel et al., 1999; Vaz et al., 1999; Wang et al., 1999], also demonstrate differences in their clinical presentations which may in part depend upon the presence of environmental stressors and their genotypic variation with our case. One boy of German descent had 10% of control carnitine uptake in fibroblasts and presented with muscle weakness from infancy, whereas cardiomegaly and impaired myocardial dysfunction were detected later at 6 yr of age [Burwinkel et al., 1999]. Another 8-yr-old boy of German descent had <10% of control carnitine uptake in fibroblasts and presented at 2 yr age with exercise intolerance and weakness with a dilated cardiomyopathy [Vaz et al., 1999]. The third child was of East Indian descent and presented at less than 2 yr of age with hypoglycemia and Reye-like episodes [Wang et al., 1999]. Our case 2 is a 15% -yr-old Croatian girl who had 1% of control carnitine uptake in fibroblasts and was hypotonic from birth. She suffered recurrent episodes of hypoglycemic coma during infections between 24 to 36 yr of age. At this time, she was also shown to have a hypertrophic cardiomyopathy.

Cases 9, 10, and 11 highlight the importance of early treatment intervention. Because of the prior diagnosis of his two older siblings, the youngest infant (case 11) was diagnosed and treated from birth with high-dose carnitine supplementation and has responded very well with no clinical manifestations of the disease. Because these three siblings have the same genotype and biochemical phenotype, the variations in their age of presentation (e.g., diagnosis of hypertrophic cardiomyopathy at 3 yr of age in case 9 and at 8 days of age in his sister, case 10) may be related to the earlier prospective investigation of the second sibling after diagnosis of the first child, rather than to an earlier-onset cardiomyopathy. The subsequent clinical course is likely related to compliance with carnitine therapy and perhaps the dose. The youngest sibling, age 3 yr, was treated from birth with an initial dose of 200 mg/kg/d, increased to 400 mg/kg/d at 7 mo of age, and has been asymptomatic. This is in sharp contrast to his symptomatic 4-yr-old sister (Case 10), treated with 100 mg/kg/d of carnitine from 9 days of age and increased to 400 mg/kg/d at 15 mo, in whom compliance has been variable, and who has persisting moderate hypertrophic cardiomyopathy. This highlights the importance of regular divided daily carnitine administration, given its rapid renal clearance from plasma, leading to significant reductions in serum carnitine 12 hr postdose.

Furthermore, no clear correlation could be established between the residual carnitine uptake (Table II) and severity of clinical presentation or age of onset (Table I). A similar observation has been reported in a study of 4 European patients [Wang et al., 2000]. This therefore suggests that the wide variability in phenotypic expression in CUD is most likely related to exogenous stressors that exacerbate the carnitine deficiency. These could include decreased intake due to dietary carnitine deficiency, drugs that increase the elimination of carnitine, and conditions such as fasting or infection, which would increase the demands on carnitine-dependent fatty acid oxidation.

On consideration of the correlation between the genotype and the residual carnitine uptake in cultured skin fibroblasts in our series of cases, the most severe biochemical defects (e.g., <5% of control carnitine uptake) appeared to be associated with homozygosity for a frameshift mutation in exon 5 (g.17081delC), resulting in a significantly truncated protein, homozygosity for a missense mutation (T440M), homozygosity for a premature (Y401X) STOP codon, and compound heterozygosity for a deletion and missplicing event (delF23/IVS3 + 1G > A). However, a larger series of cases would be required to draw definitive conclusions regarding the relative severity of the biochemical phenotype arising from these specific genotypes. The combination of the T440M missense mutation with another missense mutation (R169W) or with a frameshift mutation in exon 6 (g.19015delA) appears to partially restore carnitine uptake from 2.8% to 20%, suggesting partial genetic complementation as one possibility. This could be further investigated through the expression of specific mutant OCTN2 cDNAs generated by site-directed mutagenesis.

A review of all of the SLC22A5 (OCTN2) mutations reported to date in 27 CUD patients and 9 carriers by various groups, including the 11 mutations reported in this study, are summarized in Table III [Lamhonwah and Tein, 1998; Burwinkel et al., 1999; Koizumi et al., 1999; Nezu et al., 1999; Tang et al., 1999; Vaz et al., 1999; Wang et al., 1999; Mayatepek et al., 2000; Wang et al., 2000]. So far, mutations have been described in exons 1, 2, 3, 5, 6, 7, and 8 and in introns 3 and 8 of the OCTN2 gene. The number of mutant alleles (n = 9 in exon 1; n = 5 in exon 2; n = 7 in exon 3; n = 1 in intron 3; n = 11 in exon 5; n = 3 in exon 6; n = 3 in exon 6; n = 18 in exon 8; n = 2 in intron 8) is highest in exons 1, 3, 5, and 8. We observed a silent polymorphism in exon 1. We also found a silent polymorphism in exon 4 in which there is a higher frequency of the allele with nucleotide g.15524G (24 of 36 alleles) than the allele with nucleotide g.15524A (12 of 36 alleles). This variant allele does not change the aa Leu269.

The nine mutant alleles at R282 and two mutant alleles at W 283 in exon 5 would suggest an area of increased susceptibility for mutations. The arginine residue at position 282 and the tryptophan residue at position 283 are highly conserved in mouse, rat, and human OCTN1 and OCTN2 [Burckhardt and Wolff, 2000] and in mouse OCTN3 [Nezu, 1998, Genbank]. Other investigators have described an R282X premature stop codon due to g. 17081C > T, which leads to the production of a truncated protein shortened by 275 aa. This has been reported in three individuals of German
descent (two homozygotes and one compound heterozygote) [Burwinkel et al., 1999; Vaz et al., 1999] and in one homozygous individual of East Indian descent [Wang et al., 1999]. Because this particular mutation has been found in Asiatic Indian and German families, it has been suggested that R282X may be a recurrent mutation that has arisen in diverse genetic backgrounds or else a very ancient founder mutation [Burwinkel et al., 1999]. In all of the homozygous cases, the fibroblast carnitine uptake was reduced to 5% to 10% of controls. A decrease in mature OCTN2 mRNA to ~25% of controls has been demonstrated in one homozygote, suggesting a decrease in mRNA stability [Wang et al., 1999]. Furthermore, Wang et al. [1999] have demonstrated that expression of the mutant cDNA in CHO cells failed to increase carnitine transport significantly above the transport rate of parental CHO cells. Vaz et al. [1999] showed that transfection of the wild-type OCTN2 cDNA into mutant fibroblasts homozygous for R282X resulted in a complete restoration of carnitine uptake, confirming the identity of the disease-causing mutation. Two further mutations in this region, g.17084T>C and g.17086G>T, result in W283R [Mayatepek et al., 2000] and W283C [Koizumi et al., 1999], respectively. Both individuals, one Caucasian and one Japanese, were heterozygous for these respective mutations. Expression of these mutant cDNAs in HEK 293 cells resulted in markedly reduced carnitine uptake that was 5% and 2% of wild-type cDNA for the W283R [Mayatepek et al., 2000] and W283C [Koizumi et al., 1999] mutations, respectively. Given the recurrence of mutations in this region, this would appear to be a "hot spot" for mutations for which molecular probes would be warranted for diagnostic purposes.

Knowledge of the common mutations will facilitate the development of a panel of molecular probes for rapid diagnosis of CUD.

### TABLE III. Mutations in the High-Affinity Carnitine Transporter SLC22A5/OCTN2 Gene in 27 CUD Patients and 9 Carriers*

<table>
<thead>
<tr>
<th>Location</th>
<th>Mutation</th>
<th>Consequence of mutation</th>
<th>No. of alleles</th>
<th>Ethnic origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>g.4-5insC</td>
<td>Frameshift</td>
<td>1</td>
<td>Japanese</td>
<td>Nezu et al., 1999</td>
</tr>
<tr>
<td>(-91-22del) 113-bp del</td>
<td>Loss of first two TM domains</td>
<td>4</td>
<td>Japanese</td>
<td>Nezu et al., 1999</td>
<td></td>
</tr>
<tr>
<td>g.67-69delITTC</td>
<td>delP23</td>
<td>1</td>
<td>East Indian</td>
<td>This report</td>
<td></td>
</tr>
<tr>
<td>g.265ins/dupGGCTCGC-CACC (or g.265dup11)</td>
<td>Frameshift (I89G)</td>
<td>1</td>
<td>Unknown</td>
<td>This report</td>
<td></td>
</tr>
<tr>
<td>g.95 A &gt; G</td>
<td>N32S</td>
<td>2</td>
<td>Asian</td>
<td>This report</td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>g.8418G &gt; A</td>
<td>W132X</td>
<td>1</td>
<td>Japanese</td>
<td>Nezu et al., 1999</td>
</tr>
<tr>
<td>g.8418G &gt; A</td>
<td>W132X</td>
<td>1</td>
<td>Chinese</td>
<td>Tang et al., 1999</td>
<td></td>
</tr>
<tr>
<td>g.8418G &gt; A</td>
<td>W132X</td>
<td>3*</td>
<td>Japanese</td>
<td>Koizumi et al., 1999</td>
<td></td>
</tr>
<tr>
<td>Exon 3</td>
<td>g.14197G &gt; A</td>
<td>R169Q</td>
<td>1</td>
<td>German</td>
<td>Burwinkel et al., 1999</td>
</tr>
<tr>
<td>g.14196C &gt; T</td>
<td>R169W</td>
<td>1</td>
<td>Caucasian</td>
<td>This report</td>
<td></td>
</tr>
<tr>
<td>g.14226A &gt; T</td>
<td>M179L</td>
<td>1*</td>
<td>Japanese</td>
<td>Koizumi et al., 1999</td>
<td></td>
</tr>
<tr>
<td>g.14323A &gt; G</td>
<td>Y211C</td>
<td>2</td>
<td>Moroccan</td>
<td>Vaz et al., 1999</td>
<td></td>
</tr>
<tr>
<td>g.14323A &gt; G</td>
<td>Y211C</td>
<td>2</td>
<td>Cape Verde</td>
<td>Vaz et al., 1999</td>
<td></td>
</tr>
<tr>
<td>Intron 3</td>
<td>g.14344G &gt; A</td>
<td>IVS3+1 G &gt; A ⇒ 5' splice signal</td>
<td>1</td>
<td>Irish</td>
<td>This report</td>
</tr>
<tr>
<td>Exon 5</td>
<td>g.17081C &gt; T</td>
<td>R282X</td>
<td>2</td>
<td>East Indian</td>
<td>Wang et al., 1999</td>
</tr>
<tr>
<td>g.17081C &gt; T</td>
<td>R282X</td>
<td>3</td>
<td>German</td>
<td>Burwinkel et al., 1999</td>
<td></td>
</tr>
<tr>
<td>g.17081C &gt; T</td>
<td>R282X</td>
<td>2</td>
<td>German</td>
<td>Vaz et al., 1999</td>
<td></td>
</tr>
<tr>
<td>g.17081delC</td>
<td>Frameshift (R282D) ⇒ 294 aa protein</td>
<td>2</td>
<td>Croatian</td>
<td>This report</td>
<td></td>
</tr>
<tr>
<td>g.17084T &gt; C</td>
<td>W283R</td>
<td>1</td>
<td>Caucasian</td>
<td>Mayatepek et al., 1999</td>
<td></td>
</tr>
<tr>
<td>g.17086G &gt; T</td>
<td>W283C</td>
<td>1*</td>
<td>Japanese</td>
<td>Koizumi et al., 1999</td>
<td></td>
</tr>
<tr>
<td>Exon 6</td>
<td>g.19015delA</td>
<td>Frameshift (T337P) ⇒ 347 aa protein</td>
<td>3</td>
<td>Turkish</td>
<td>This report</td>
</tr>
<tr>
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<td>g.20876-20877insA</td>
<td>Y401X</td>
<td>2</td>
<td>Caucasian</td>
<td>This report</td>
<td></td>
</tr>
<tr>
<td>Exon 8</td>
<td>g.22521 C &gt; T</td>
<td>T440M</td>
<td>3</td>
<td>Turkish</td>
<td>This report</td>
</tr>
<tr>
<td>g.22521 C &gt; T</td>
<td>T440M</td>
<td>2</td>
<td>Croatian</td>
<td>This report</td>
<td></td>
</tr>
<tr>
<td>g.22538G &gt; T</td>
<td>V446F</td>
<td>1</td>
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<td>Mayatepek et al., 1999</td>
<td></td>
</tr>
<tr>
<td>g.22556G &gt; A</td>
<td>E452K</td>
<td>2</td>
<td>Pakistani</td>
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<td></td>
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<tr>
<td>g.22506delG</td>
<td>458X</td>
<td>1</td>
<td>Caucasian</td>
<td>Wang et al., 1999</td>
<td></td>
</tr>
<tr>
<td>g.22602C &gt; G</td>
<td>S467C</td>
<td>4*</td>
<td>Japanese</td>
<td>Koizumi et al., 1999</td>
<td></td>
</tr>
<tr>
<td>Intron 8</td>
<td>g.23713G &gt; A</td>
<td>IVS8-1G &gt; A ⇒ 3' splice signal</td>
<td>2</td>
<td>Japanese</td>
<td>Nezu et al., 1999</td>
</tr>
<tr>
<td>cDNA</td>
<td>c.34-1428del</td>
<td>92 aa protein</td>
<td>1</td>
<td>Italian</td>
<td>Lamhonwah and Tein, 1998</td>
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<tr>
<td>cDNA</td>
<td>c.34-1428del</td>
<td>92 aa protein</td>
<td>1</td>
<td>Mexican</td>
<td>Lamhonwah and Tein, 1998</td>
</tr>
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</table>

*Carrier.
diagnosis. This will promote early treatment intervention, reducing the morbidity and mortality in this lethal but eminently treatable disorder, and will facilitate genetic counseling in these families. Identification of heterozygotes for OCTN2 mutations will also help to identify individuals at risk for late-onset benign cardiac hypertrophy [Koizumi et al., 1999], which may be exacerbated by other cardiac risk factors. Examination of the impact of these mutations on the functional characteristics of the high-affinity carnitine transporter will allow direction of specific site-directed mutagenesis experiments to elucidate the critical structure–function relationships of the OCTN2 protein, with respect to its key functional sites such as the carnitine binding site, sodium channel, and sulfhydryl-binding sites. Expression studies of a GFP-mutant OCTN2 cDNA in mammalian cells will enable assessment of the impact of several transmembrane and truncating mutations on the intracellular localization of the mutant protein. Increased knowledge of the specific functional derangements associated with individual mutations may foster the development of more targeted therapeutic approaches to enhance carnitine delivery to cells. Overall, these studies should contribute to the fundamental understanding of the disease pathogenesis in the plasmalemmal high-affinity carnitine transporter defect.

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