MUTATION IN BRIEF

Two Novel Missense Mutations of the OCTN2 Gene (W283R and V446F) in a Patient with Primary Systemic Carnitine Deficiency

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Primary systemic carnitine deficiency (SCD) is an autosomal recessive disorder of fatty acid oxidation caused by defective cellular carnitine transport. The disease is characterized by metabolic derangement simulating Reye´s syndrome, hypoglcaemia, progressive cardiomyopathy and skeletal myopathy. Recently, it was shown that SCD is caused by mutations in the organic cation/carnitine transporter OCTN2 (SLC22A5). We report two novel mutations, W283R and V446F, which are both missense mutations in an affected infant. In vitro expression studies demonstrated that both are actually function-loss mutations with virtually no uptake activity. This is the first report of compound heterozygosity for two missense mutations in a patient with SCD. © 1999 Wiley-Liss, Inc.

KEY WORDS: primary systemic carnitine deficiency; SCD; OCTN2; SLC22A5

INTRODUCTION

Primary systemic carnitine deficiency (SCD; MIM# 212140) is a potentially acute lethal, autosomal recessive disorder characterized by progressive cardiomyopathy, skeletal myopathy, hypoketotic hypoglycaemia and hyperammonaemia (Karpati et al. 1975; Treem et al. 1988; Roe & Coates 1995).

Studies of fibroblasts from affected patients established that the primary defect in SCD involves a defect in the sodium-dependent high-affinity transporter situated in the plasmalemmal membrane (Tein et al. 1996; Treem et al. 1988). The same transporter is also involved in the renal reabsorption of carnitine thus explaining the excessive renal waste of carnitine in SCD patients.

A human cDNA encoding a sodium-dependent, high affinity carnitine transporter was recently cloned from human kidney (Tamai et al. 1998) and mapped to chromosome 5q31 (Wu et al. 1998). The gene involved, called OCTN2, (SLC22A5; MIM# 603377) was a good candidate for the gene mutated in SCD. In the meantime, there

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appeared the first reports of mutations in the OCTN2 gene which were shown to be causative in SCD-patients (Nezu et al. 1999; Tang et al. 1999; Wang et al. 1999; Burwinkel et al. 1999).

We report two novel causative mutations, W283R and V446F, which are both missense mutations causing SCD in an affected infant.

PATIENT AND METHODS

Patient History

The male patient was born as the first child in a Caucasian family. At the age of 6 months during an episode of an upper airway infection he was admitted comatous with acute metabolic derangement and a Reye-like syndrome including encephalopathy, hypoketotic hypoglycaemia, elevated liver enzymes and steatosis. Serum free carnitine was 1 µmol/l (controls 22-50 µmol/l) with a normal long-chain acylcarnitine. After intensive care treatment and administration of high doses of carntine (initially intravenously, later on orally with a dose of 200 mg/kg/day) he rapidly recovered. At present, he is 10 months of age and his neurological development is appropriate for age. There are no signs of cardiac or skeletal muscle involvement.

Mutation Analysis

All 10 exons of OCTN2 gene, SLC22A5, were amplified by PCR from genomic DNA purified from whole blood using following primers: exon 1 (952bp), OCN2 43 (5'-GCAGGACCAAGG-CGGCGGTGTCAG-3') and OCN2 44 (5'-AGACTAGAGGAAAAACGGGATAGE.3'); exon 2 (823bp), OCN2 25 (5'-AGATTTTTAGGAGCAAGEGTTAGA-3') and OCN2 26 (5'-GAGGCA-GACACCGTGGCACTACTA-3'); exon 3and 4 (1500bp), OCN2 27 (5'-TTCACACCCACT-TAC-TGGATGGAT-3') and OCN2 50 (5'-ATTCTGTTTTGTTTTGGCTCTTTT-3'); exon 5 (1039bp), OCN2 31 (5'-AGCAGGGCCTGGGCTGACATAGAC-3') and OCN2 32 (5'-AAA-GGACCT-GACTCCAAGATGATA-3'); exon 6 (480bp), OCN2 33 (5'-TCTGACCACCTC-TTCTTCCCA-TAC-3') and OCN2 34 (5'-GCCTCCTEAGCCACTGTCGGTAAC-3'); exon 7 (1066bp), OCN2 35 (5'-ATGTTGTTCCTTTTGTTATCTTAT-3') and OCN2 36 (5'-CTTGTT-TTCTTGTGTATC-GTTATC-3'); exon 8 and 9 (1558bp), OCN2 37 (5'-TATGTTTGTTTT-GCTCTCAATAGC-3') and OCN2 40 (5'-TCTGTGAGAGGGAGTTTGCGAGTA-3'); exon 10 (1158bp), OCN2 41 (5'´-TACGACCGCTTCCTGCCCTACATT-3') and OCN2 42 (5'-TCA-TTCTGCTCCATCTTC-ATTACC-3'). The PCR products were analysed by direct sequencing (Nezu et al. 1999).

In Vitro Expression

The effects of the two OCTN2 mutations, W283R and V446F, on the carnitine transport activity of OCTN2 were evaluated by in vitro expression studies as described in detail (Nezu et al., 1999; Tamai et al., 1998).

Using the original construct (pcDNA3/OCTN2) as template, two mutations were introduced by in vitro site-directed mutagenesis using the GeneEditor™ system (Promega), in accordance with the procedure suggested by the manufacturer. Following mutagenic oligonucleotides were hybridized to single-stranded pcDNA3/OCTN2 plasmid DNA with the selection oligonucleotide (bottom strand), and mutant strands were synthesized. After two cycles of GeneEditor™ antibiotic selection, the resistant clones were picked up, and the mutations were confirmed by sequencing.

[Trp283Arg]
OBM W283R
5’-GAGTCCCCCCGACGGCTCATCTCTCAG-3’

[Val446Phe]
OBM V446F
5’-GCCTTTTCCATGTTCTACGTGTACACA-3’

The wild type and mutant expression plasmid were transfected to HEK 293 cells by a calcium phosphate precipitation method. The cells were cultivated for 48 hrs and subjected to transport assay. The harvested cells were thereafter incubated with [^{3}H]carinitine (10 nM) for 3 min at 37°C and amount of radioactivity in the cells

was measured. Uptake by Mock cells that were transfected with pcDNA3 vector alone was used as blank. The transport activity of Mock cell is indicated in Table 1.

RESULTS

Mutational analysis identified two mutations in this infant with primary carnitine deficiency. The patient inherited a missense mutation in exon 5 from his father. There was a T -> C transition at nucleotide position (nt) 7305 which alterates codon 283 W (TGG) to R (CGG). In addition, we found a second missense mutation in exon 8 inherited from his mother. This was a G -> T transition at nt 22759 which alters codon 446V (GTC) to F (TTC). The nucleotide positions of OCTN2 are according to GenBank accession no. AB016625 (human OCTN2 gene). Codon numbers are according to GenBank entry no. AB015050 (human OCTN2 cDNA).

Results of further analysis including sequencing of the complete coding sequence eliminated the possibility of the presence of other mutations.

The effects of the two mutations, W283R and V446F, on the transport function of OCTN2 revealed that these mutations lead to a completely deficient cellular carnitine transport activity (Table 1).

Table 1. Carnitine uptake function of the wild-type, Mock (pcDNA3 vector alone), W283R and V446F. Results are expressed as mean±S.E. of three determinations.

Cell type	Transport activity for L-carnitine (pmol/mg protein/3 min)
Wild type	7.65 ± 0.14
Mock (pcDNA3 vector alone)	0.173 ± 0.004
W283R	0.392 ± 0.015
V446F	0.215 ± 0.005

DISCUSSION

Direct sequence analyses have identified two unique mutations in an infant with SCD, a T -> C base change at codon 283 (W283R), and a G -> T baee change at codon 446 (V446F). This means that the patient is compound heterozygous of these novel mutations. We have not identified these mutations in other patients with SCD.

Up to now, a total number of 10 mutations have been reported in 9 different patients (Nezu et al., 1999; Tang et al. 1999; Wang et al. 1999; Burwinkel et al. 1999). Some of them were homozygous for either a deletion containing the start codon, splice-site mutation, or a single base pair substitution. The other patients were compound heterozygous for mutations causing a frameshift, premature stop codon, deletion or truncation.

Our infant represents the first case of a SCD patient with two unreported missense mutations: W283R and V446F. Expression studies with constructed expression plasmids for these mutants, demonstrated that both are actually function-loss mutations with virtually no uptake activity. The vicinity of codon 283 may be a mutation hotspot, as a mutation in codon 282 has been found repeatedly (Wang et al. 1999; Burwinkel et al. 1999).

In summary, our data clearly underline the fact that mutations in the OCTN2 gene are responsible for SCD. The spectra of mutations in this gene seems to be variable. However, mutation analysis already represents a new tool for reliable prenatal and postnatal diagnosis of SCD.

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