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Analysis of genetic mutations in Chinese patients with systemic primary carnitine deficiency

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ABSTRACT

Systemic primary carnitine deficiency (CDSP) is caused by mutations in *SLC22A5* gene, which encodes organic cation transporter 2(OCTN2). CDSP leads to skeletal or cardiac myopathy and hepatic encephalopathy. The present study aimed to identify *SLC22A5* gene mutations and analyze the potential relationship between genotype and clinical symptoms in 20 Chinese patients with CDSP. The complete coding region of the *SLC22A5* gene including intron-exon boundaries were amplified and sequenced in all patients. Eighteen different mutations were found; of which, nine were novel. The mutations clustering in exons 1 and 4 accounted for 66.7% of all mutant alleles (26/39). The c.760C>T (p. R254X) was the most frequent mutation (25.6%, 10/39), suggesting it as an ethnic founder mutation. The relationship between genotype and phenotype was investigated in patients carrying the R254X mutation. Homozygous patients with R254X were late-onset cases who presented with dilated cardiomyopathy and muscle weakness after 1 year of age. Compound heterozygous patients carrying R254X, combined with other missense mutations occurred in very specific positions, dramatically altered OCTN2 protein function. Based on the analysis of case studies, a clear relationship between free carnitine (C0) level in plasma and OCTN2 genotype was not found in the present work, however, the low plasma C0 level could not indicate disease severity or genotype. Further functional studies with a large sample size are required to understand the relationship between R254X mutation and CDSP.

1. Introduction

Systemic primary carnitine deficiency (CDSP, MIM 212140) is an autosomal recessive disorder of fatty acid oxidation caused by defective activity of organic cation transporter 2 (OCTN2). The disease is characterized by very low blood levels of free carnitine (C0) and other acylcarnitines [Roe and Coates, 1995; Scaglia and Longo, 1999]. Carnitine is essential for transferring long-chain fatty acids from cytosol to mitochondria for subsequent beta oxidation. Carnitine deficiency in infants during their early life leads to an acute metabolic presentation with symptoms such as hypoketotic hypoglycemia, Reye syndrome, and sudden death. Late onset symptoms involve three systems such as cardiac muscle, skeletal muscle, and central nervous system, which are respectively affected by progressive cardiomyopathy, myopathy, and encephalopathy due to hypoketotic hypoglycemia and hyperammonemia. The mean age of CDSP onset ranges from 1 month to 7 years [Longo et al., 2006], although a few patients have remained completely asymptomatic into adulthood [Spiekerkoetter et al., 2003; Wang et al., 2001]. CDSP is diagnosed by measuring plasma levels of C0 and acylcarnitines using tandem mass spectrometry (MS/MS) [Koizumi et al., 1999]. Both of these biomarkers are extremely reduced in patients with CDSP (i.e., <10% of controls) [Scaglia and Longo, 1999]. CDSP occurs in 1:40,000 people in Japan [Koizumi et al., 1999] and 1:120,000 in Australia [Wilcken et al., 2003]. The frequency in the United States (US) and Europe has not been defined; however, the prevalence can be estimated to be approximately 1:50,000 in the US on the basis of newborn screening data and reported cases (GeneReviews® [Internet]). Few reports regarding the prevalence of CDSP in China have been published so far.

Abbreviations: C0, free carnitine; CDSP, systemic primary carnitine deficiency; MS/MS, tandem mass spectrometry; OCTN2, organic cation transporter 2.

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Gene for CDSP (*SLC22A5*, MIM# 603377) encodes OCTN2 and maps to chromosome 5q31. To date, more than 100 mutations have been reported in the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php). OCTN2 is a transmembrane carnitine transporter comprising 557 amino acids that contain 12 transmembrane domains and one adenosine triphosphate-binding domain. Although no common mutations have been found, recurrent mutations in *SLC22A5* have been reported in several populations. Recent evidence suggested that there were a few ethnic-specific founder mutations in *SLC22A5*. For example, the truncated mutation R254X has previously been described in three symptomatic Chinese patients and two of 250 healthy controls [Tang et al., 2002]. This variant has been suggested as a founder mutation in the Chinese population [Tang et al., 2002]. It was also considered as a recurrent or very ancient founder mutation in the Saudi Arabian population, after it was reported in one family [Lamhonwah et al., 2004]. Another mutation, S467C, is believed to be an ancient founder mutation in the Akita Japanese population [Koizumi et al., 1999]. W132X and W283C mutations are also common in East Asian populations [Koizumi et al., 1999; Nezu et al., 1999; Tang et al., 1999], and the R282X mutation is common in Caucasians [Burwinkel et al., 1999; Vaz et al., 1999; Wang et al., 1999]. Despite the extensive works to identify founder mutations globally, previous studies have not found a definite correlation between genotype and clinical presentation of CDSP [Lamhonwah et al., 2002; Wang et al., 2001, 2000, 1999]. Moreover, there have been very few reports on the relationship between mutations and CDSP in the Chinese population [Korkmaz et al., 2005; Tang et al., 1999, 2002]. Therefore, the present study investigated the spectrum of *SLC22A5* gene mutations in 20 Chinese patients and attempted to investigate the relationship between genotype and phenotype in a limited number of patients using a case study approach.

2. Materials and methods

2.1. Ethics statement

This study was approved by the local Institutional Review Board, and written informed consent was obtained from each study participant or their parents.

2.2. Patient selection and metabolite detection

Fifteen unrelated symptomatic patients with CDSP and five asymptomatic patients (detected using MS/MS through a newborn screening program) were enrolled between 2008 and 2011. Newborns with C0 levels of <10 μmol/L were retested to confirm the diagnosis of CDSP by measuring C0, cardiac enzymes, glucose, ammonia, and lactic acid in blood. The patients also underwent electrocardiography, urinary organic acid analysis, urine ketones, and genetic sequencing. Measurements of C0 and acylcarnitines in blood and genetic analyses were performed in all patients and their parents. The patients diagnosed with CDSP received treatment with oral L-carnitine (100–400 mg/kg/day) and acetylcarnitine (50–100 mg/kg/day) orally. Treatment response was assessed by monitoring clinical symptoms and measuring levels of C0 and acylcarnitines in dried blood spots with MS/MS (Applied Biosystems, API 2000, Foster City, CA, USA) [Han et al., 2007, 2008; Komlosi et al., 2009]. The level of organic acids in urine was measured by gas chromatography-MS (Shimadzu Limited, Tokyo, Japan, QP2010). Sample preparation and detection procedures were based on previously reported methods [Kuhara, 2002]. The phenotype and genotype details of all the enrolled patients are listed in Tables 2 and 3.

2.3. Deoxyribonucleic acid (DNA) sequencing analysis

Genomic DNA was extracted from peripheral blood leukocytes of all 20 patients with CDSP and their parents. The entire coding sequence of the *SLC22A5* gene, including 10 exons and their exon/intron boundaries, was amplified by polymerase chain reaction and analyzed on an ABI3700 automated DNA sequencer (Applied Biosystems). The sequencing results were compared to the GenBank *SLC22A5* sequence (NM_003060.3). The mutations found in the patients were confirmed with that of the parents. If the mutations had not previously been reported, they were compared to 100 normal controls to exclude polymorphisms. The structural/functional domains of the OCTN2 protein were identified using the Universal Protein Resource (http://www.uniprot.org/). Detailed information regarding the primers would be available upon request.

3. Results

3.1. Clinical presentation

All 20 patients had decreased blood levels of C0 and acylcarnitines compared to the reference range (10–60 μmol/L). The asymptomatic patients enrolled through the newborn screening program had a mean age of 11.5 months and ranged from 0.7 months (21 days) to 54 months old (Table 1). These patients had a C0 level of 4.16 ± 1.77 μmol/L (mean ± standard deviation). The five infants recruited through newborn screening program had no symptoms (Table 2). However, three patients had an affected sibling who had died, and one of these three patients died from dyspnea at 6 months of age. In contrast, the symptomatic patients presented with a lower C0 of 1.75 μmol/L. The symptomatic patients presented with a variety of clinical symptoms (Table 3), which included muscle weakness (7/15), dilated cardiomyopathy (12/15), hepatomegaly (4/15), encephalopathy (3/15), sudden infant death (1/15), feeding difficulty (1/15), recurrent pneumonia (1/15), vomiting (2/15), abdominal pain (1/15), and diarrhea (1/15) (Table 3).

Routine laboratory tests revealed that liver enzymes were elevated concomitantly with hepatomegaly. The average plasma

Table 1
Clinical description of enrolled patients.

	Asymptomatic	Symptomatic
Number of patients	5	15
Age months (range)	11.5 (0.7–54)	25.8 (6–72)
Free Carnitine μmol/L (standard deviation)	4.16 (1.77)	1.75 (1.45)
Alleles [% of patients (N)]:[a]		
c.51C>G (p. F17L)	60 (3)	13 (2)
c.396G>A (p. W132X)	20 (1)	–
c.760C>T (p. R254X)	–	53 (8)
c.745_748delTTTG[a] (p. F249LfsX14)	–	6 (1)
c.505C>T (p. R169W)	–	6 (1)
c.700G>C[a] (p. G234R)	–	6 (1)
c.338G>A[a] (p. C113Y)	20 (1)	20 (3)
c.433dupA[a] (p. T145AsnfsX50)	–	6 (1)
c.1195C>T (p. R399W)	20 (1)	–
c.248G>T (p. R83L)	20 (1)	–
c.797C>T (p. P266L)	40 (2)	–
c.1400C>G (p. S467C)	20 (1)	–
c.497+1G>T[a]	–	13 (2)
c.517 del C[a] (p. L173CysfsX)	–	6 (1)
c.1372delG[a] (p. V458X)	–	6 (1)
c.865C>T (p. R289X)	–	6 (1)
c.1412G>A (p. R471H)	–	6 (1)
c.393+5 G>A[a]	–	6 (1)
Not detected	–	6 (1)

[a] Novel mutations identified.