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SHORT REPORT

Prenatal diagnosis of a novel mutation, c.529C>T (p.Q177X), in the *BCKDHA* gene in a family with maple syrup urine disease

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Summary Maple syrup urine disease (MSUD) is an autosomal recessive metabolic disorder caused by defective activity of the branched-chain α-keto-acid dehydrogenase (BCKD) complex. The disease-causing mutations can affect the *BCKDHA*, *BCKDHB* or *DBT* genes encoding for the E1a, E1b, and E2 subunits, respectively, of the BCKD complex. Here we report a girl who first presented to our clinic at 4 years of age with profound mental retardation. A diagnosis of MSUD was subsequently made based on the results of plasma amino acid analysis. Mutation analysis confirmed that she was homozygous for a novel mutation, c.529C>T (p.Q177X) in *BCKDHA*, while both parents, who were first cousins, were heterozygous. This enabled us to give an option of prenatal diagnosis to the parents. The prenatal testing for MSUD was performed during the mother's subsequent pregnancy and revealed that the fetus was heterozygous for the mutation. The healthy male neonate was born and his genotype was tested by restriction enzyme analysis, which confirmed the result of the prenatal testing. In summary, a late diagnosis of MSUD in patients without an unusual odour could occur especially in countries without neonatal screening programs as seen in the index patient. Mutation detection was, however, still beneficial to the family since prenatal testing could be performed in subsequent pregnancies. In addition, a novel mutation was found, expanding the mutation spectrum of this disease.

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Abbreviations

BCAA	branched-chain amino acids
BCAT	branched-chain aminotransferase isozyme
BCKD	branched-chain α-keto acid dehydrogenase
MRI	magnetic resonance imaging
MSUD	maple syrup urine disease
RFLP	restriction fragment length polymorphism

Introduction

Maple syrup urine disease (MSUD; OMIM 248600) is an autosomal recessive metabolic disorder caused by defective activity of the branched-chain α-keto-acid dehydrogenase (BCKD) complex which catalyses the catabolism of the branched-chain amino acids (BCAA): leucine, isoleucine, and valine (Zhang et al 1991). The BCKD is a multimeric complex comprising three catalytic components: a branched-chain α-keto-acid decarboxylase (E1), a dihydrolipoyl transacylase (E2), and a

dihydrolipoamide dehydrogenase (E3). E1 is a heterotetrameric complex consisting of two E1α and two E1β subunits encoded by the *BCKDHA* and *BCKDHB* genes, respectively. E2 and E3 are encoded by the *DBT* and *DLD* genes, respectively. BCKD complex deficiency leads to accumulation of the corresponding amino acids and marked ketoacidosis with clinical features of neurological abnormalities and mental retardation. Patients usually have a distinctive maple syrup odour in their urine. Mutations causing MSUD are genetically heterogeneous and have been identified in the *BCKDHA*, *BCKDHB*, and *DBT* genes (Danner and Doering 1998; Nellis and Danner 2001).

Based on the clinical presentation and biochemical responses to thiamine administration, MSUD can be divided into four types: classic, intermediate, intermittent, and thiamine-responsive. Most individuals affected with MSUD suffer from the severe classic form with residual BCKD activities less than 2% of those of normal subjects. If left untreated, the prognosis is poor as the majority of patients die within the first few months of life, usually from a metabolic crisis and neurological deterioration (Chuang and Shih 2001). Newborn-screening programmes in some countries lead to early diagnosis and specific treatment.

Here we report a 4-year-old girl with MSUD caused by a novel mutation in the *BCKDHA* gene. This finding enabled us to provide appropriate genetic counselling and successful prenatal diagnosis during subsequent pregnancy.

Patient

The index patient was born at term in India to a 22-year-old, gravida 1, para 0, Indian mother and a 22-year-old Indian father. The parents were first cousins. The pregnancy and labour were uneventful. Her birth weight was 3500 g. At 5 days of age, she developed seizures and dystonia. Brain magnetic resonance imaging (MRI) at 1 week of age suggested generalized delayed myelination. EEG at 8 months of age demonstrated severe epileptic cerebral dysfunction. Seizures ceased at the age of 9 months, but the patient continued to vomit every day. Her development was at best at 6 months of age, when she started holding her head. She had regular follow-up visits in India and was totally dependent on the caregiver, but was not on any medications and had not been hospitalized. The diagnosis was made at 4 years of age when the family came to Thailand and she was referred to our hospital. She presented with profound mental retardation. Her head circumference was 45.5 cm (−3 SD) and her weight was 14 kg (−1 SD). She had hypertonia and hyperreflexia with positive clonus. Neither dysmorphic features nor abnormal odour was observed. Plasma amino acid analysis revealed increased levels of branched-chain amino acids (1542 μmol/L for leucine, 506 μmol/L for valine, and 376 μmol/L for isoleucine). Urine organic acid analysis by gas chromatography–mass spectrometry revealed a large amount of 2-hydroxyisovaleric acid. The clinical picture and the greatly elevated BCAA concentrations in plasma indicated that the patient suffered from a severe form of MSUD. Treatment with a special formula was subsequently started. She responded poorly to the treatment. Her developmental status remained the same. She also suffered from multiple episodes of pneumonia, and died at the age of 7 years.

Mutation analysis by PCR-sequencing revealed a novel homozygous mutation, c.529C>T, in exon 5 of the *BCKDHA* gene (data not shown). The nucleotide change was predicted to cause a truncating mutation (p.Q177X) in the E1α subunit of the BCKA complex. Using restriction fragment length polymorphism (RFLP) analysis, it was confirmed that the index patient was homozygous for the c.529C>T transition and both parents were found to be carriers of the mutation (Fig. 1).

Identification of the disease-causing mutation enabled us to give more accurate genetic counselling. One year

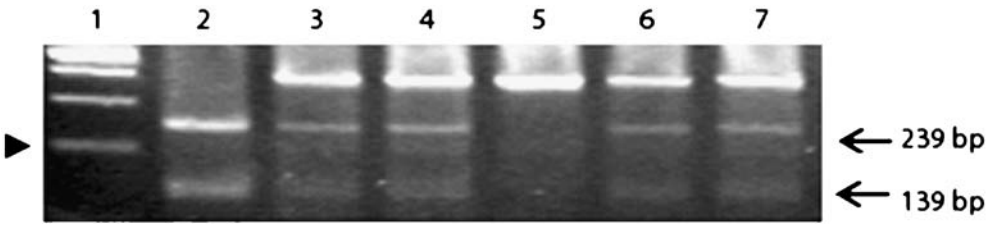

Fig. 1 PCR-RFLP analysis of *BCKDHA* c.529C>T. Lane 1, 100 bp marker; lane 2, unaffected control; lane 3, father; lane 4, mother; lane 5, index patient; lane 6, second child (fetal CVS); lane 7, second child (born, peripheral blood). In lane 2, *Sau96*I digested the wild-type allele of the control into 239 bp and 139 bp products (arrows). The c.529C>T mutation in the index patient eliminates the restriction site, leaving the uncut 328 bp product. The analysis showed that the index patient was homozygous for the mutation, while her parents and brother were heterozygotes. The 200 bp band is indicated by an arrow head

Table 1 Oligonucleotides and PCR conditions for MSUD mutation analysis

Name	Primer sequences for PCR 5′ to 3′	Annealing temperature (°C)
BCKDHA-F	CTGAGTGGTTGTTAGCCAAG	61
BCKDHA-R	AGACAGTGGTGTGCTGTCAG	
DBT-F	GTTGTCATTTCCGGGGTAAG	55
DBT-R	CCCAGGAGAACCATTACACC	
DLD-F	CAGCGGAGGTGAAAGTATTG	61
DLD-R	GTTCAGGAATGTGACTTC	
Ex5-F	CCTGTCTGCCTGCCAGCATG	57
Ex5-R	AGGGCTCTAGTGGTGTCACC	

after the causative mutation was found in the proband, her parents decided to have another child. Prenatal diagnosis was successful in this family.

Materials and methods

Mutation analysis

Total RNA was isolated from white blood cells of the index patient using QIAamp RNA blood mini kit (Qiagen, Valencia, CA, USA). Reverse transcription was performed using ImProm-II reverse transcriptase (Promega, Madison, WI, USA). PCR amplification of the entire coding region of the *BCKDHA*, *DBT*, and *DLD* genes was performed on the cDNA using primers specific for each gene (Table 1). PCR products were directly sequenced.

Genomic DNA was obtained from venous (whole) blood of the index patient, the parents, and the newborn. For prenatal diagnosis, genomic DNA was extracted from chorionic villi (obtained in the 11th week of gestation) and cultured amniocytes (obtained by amniocentesis in the 16th week of gestation). For mutation analysis by PCR-RFLP, exon 5 of the *BCKDHA* gene was amplified using primers Ex5-F and Ex5-R (Table 1), then treated with *Sau96*I (New England Biolabs, Beverly, MA, USA). DNA extraction and restriction enzyme digestion were performed according to the manufacturer's recommendations.

Results

Mutation analysis of the *BCKDHA* gene was first performed in the index patient and revealed a novel homozygous nonsense mutation, c.529C>T (p.Q177X). Both parents were found to be carriers of the mutation. Prenatal testing of fetal materials by RFLP analysis revealed that the fetus was heterozygous for the mutation (Fig. 1). The mother decided to continue her pregnancy, which resulted in a birth of a healthy male child. The newborn's genotype was tested by RFLP, which confirmed the prenatal testing result (Fig. 1). Amino acid and acylcarnitine levels analysed by tandem mass spectrometry were within normal limits.

Discussion

At least 100 different mutations causing MSUD have been described. Of these, 39 are reported in the *BCKDHA* gene, with the majority being missense/nonsense mutations (The Human Gene Mutation Database http://www.hgmd.cf.ac.uk, accessed July 2008). This study describes a patient with a severe form of MSUD. A novel nonsense mutation (c.529C>T; p.Q177X) was found and predicted to create a premature termination codon of the E1α subunit (NP_000700), which results in either mRNA degradation through nonsense-mediated mRNA decay process or truncation of the protein at amino acid 176, thus deleting 269 amino acids at its carboxy terminus. Both cases can cause a major disruption to the protein function.

Although the diagnosis was delayed in the index patient and she was not given the specific treatment in time to prevent further neurological damage, identification of the causative mutation was still beneficial to her family. It did not discourage her parents from having another child and allowed fetal molecular analysis.

Untreated individuals with severe MSUD usually suffer from neurological damage as seen in our patient. Early diagnosis and treatment can reduce morbidity, mortality, and length of hospitalization (Mitsubuchi et al 2005; Padilla et al 2001). Newborn screening by tandem mass spectrometry can be used to detect elevated BCAA concentrations in the blood samples, making early diagnosis and intervention possible in pre-symptomatic newborns (Chace et al 1995; Heldt et al 2005; Simon et al 2005). This can result in benign neonatal courses, normal growth, and low hospitalization rates in classic MSUD patients (Morton et al 2002). Without newborn screening, diagnosis of MSUD is

usually made between 10 and 20 days of life, when the patients become symptomatic and neurological damage has already occurred. Therefore, countries currently without a newborn screening programme for metabolic disorders should take this issue seriously into consideration.

In summary, we report a novel protein-truncating mutation in the *BCKDHA* gene in a girl with severe MSUD. Identification of the causative mutation enabled us to make a successful prenatal diagnosis in this family in which the fetus was found to be heterozygous for the mutation. This study also re-emphasizes the importance of early diagnosis and treatment of MSUD in affected individuals to help them survive and develop normally.

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