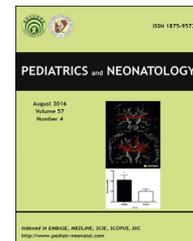


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Original Article

# Two novel mutations in the *BCKDHB* gene that cause maple syrup urine disease

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## Key Words

BCAA;  
BCKD;  
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gene mutation;  
maple syrup urine  
disease

**Abstract** *Background:* Maple syrup urine disease (MSUD) is a rare metabolic disorder of autosomal recessive inheritance caused by decreased activity of branched-chain  $\alpha$ -ketoacid dehydrogenase complex (BCKD). **Mutations in the three genes (*BCKDHA*, *BCKDHB* and *DBT*) are associated with MSUD.** Here, we describe the presenting symptoms, clinical course and gene mutation analysis of a Chinese boy with MSUD.

*Methods:* Plasma amino acid analysis was performed by tandem mass spectrometry and the levels of organic acids in urine were measured with gas chromatography-mass spectrometry. The *BCKDHB* gene was sequenced by Sanger method. Furthermore, the significance of the novel mutations was predicted by Polyphen and Mutationtaster. After diagnosis, the patient was fed with protein-restricted diet to reduce intake of BCAA and was treated with L-carnitine. Metabolic parameters, clinical presentation and mental development were followed up.

*Results:* The patient was diagnosed as MSUD. Two novel *BCKDHB* mutations (c.523 T > C and c.478-25\_552del100) were identified. In silico analysis predicted that the two mutations were "disease causing". The boy tolerated the treatment well and had symptomatic improvement. He presented with mild hypotonia and had nearly normal DQ scores at the age of 10 months. The two novel mutations resulted in the clinical manifestations of MSUD. Our results may reflect the heterogeneity of the pathogenic variants found in patients with MSUD.

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**Abbreviations:** MSUD, Maple syrup urine disease; BCKD, branched-chain  $\alpha$ -ketoacid dehydrogenase; BCKA, branched-chain hydroxyacids and ketoacid; BCAA, branched-chain amino acid; DQ, developmental quotient; MRI, magnetic resonance imaging; DWI, diffusion-weighted imaging.

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## 1. Introduction

Maple syrup urine disease (MSUD) is a rare metabolic disorder of autosomal recessive inheritance caused by decreased activity of branched-chain  $\alpha$ -ketoacid dehydrogenase complex (BCKD). BCKD complex catalyzes the oxidative decarboxylation of the branched-chain keto acids in the second step from pathway of branched-chain amino acids (BCAAs), including leucine, isoleucine and valine.<sup>1,2</sup> Deficiency of the activity of BCKD complex leads to the accumulation of BCAAs and their  $\alpha$ -keto acids in the plasma, urine and cerebrospinal fluid.<sup>1</sup> BCKD complex is comprised of four subunits of E1 $\alpha$ , E1 $\beta$ , E2 and E3, which are encoded by *BCKDHA*, *BCKDHB*, *DBT* and *DLD* genes, respectively.<sup>3</sup> Traditionally, the phenotype of MSUD is classified into five types: classic, intermediate, intermittent, thiamine-responsive and E3 deficient.<sup>4,5</sup> Classic MSUD (75% of cases) is characterized by BCKD complex activity ranging from 0% to 2% and it exhibits the most severe phenotype. In the classic MSUD, clinical onset usually occurs within the first week after birth, including maple syrup odor, poor feeding, acute metabolic decompensation and drowsiness, followed by progressive coma, seizures and even central respiratory failure. Brain edema is a common complication of metabolic decompensation.<sup>6,7</sup> If not treated urgently, this condition results in irreversible brain damage or death. The intermediate type (3–8% of normal enzyme activity) is manifested with progressive mental retardation and developmental delay.<sup>4</sup> The patients with intermittent type have normal intelligence and development until a stress situation such as infection. In thiamine-responsive type, the patients are thiamine-responsive in which administration of pharmacological dose of thiamine retrieves normal enzyme activity. In the last type, patients with E3 deficiency show a combined dysfunction of the three ketoacid dehydrogenase complexes.<sup>4</sup> MSUD is diagnosed by the presence of clinical features, elevated BCAA in plasma, and branched-chain hydroxyacids and ketoacids (BCKAs) in urine.<sup>8,9</sup>

The world-wide incidence of MSUD is estimated to be 1 in 185,000.<sup>10,11</sup> To date, over 200 mutations have been detected among *BCKDHA*, *BCKDHB* and *DBT* (Human Gene Database, <http://www.hgmd.cf.ac.uk>).<sup>6</sup> The genomic changes that impair BCKD activity can occur in any of the three genes above. In this study, we reviewed the clinical presentation, biochemical features, genetic mutations, treatment and follow-up of a case with MSUD. Furthermore, two novel mutations of the *BCKHB* gene were identified in the patient.

## 2. Methods

### 2.1. Patient and diagnosis

The patient was from Jinan Maternal and Child Care Hospital, Shangdong province. Plasma amino acid analysis was performed by tandem mass spectrometry. To further confirm the diagnosis, the levels of organic acids in urine were measured with gas chromatography-mass spectrometry. All tests were performed as routine clinical and biochemical investigation in accordance with the ethical principles of the Declaration of Helsinki. Informed consent was obtained from parents of the patient in the study.

### 2.2. DNA sequence analysis

Genomic DNA was extracted from peripheral blood leukocytes of the patient using phenol-chloroform method. The exons and flanking intronic regions of the *BCKDHB* gene were amplified by polymerase chain reaction.<sup>12</sup> Purified PCR products were sequenced. Mutation analysis was performed using the normal *BCKDHB* genomic DNA (NM\_183050) sequence as a reference.

### 2.3. Treatment and follow-up

The patient was fed with protein-restricted diet and treated with L-carnitine. Changes in the levels of BCAAs were monitored strictly once per week. The developmental quotient (DQ) was evaluated using the Revised Gesell Developmental Evaluation for children (<4 years), which provides a developmental profile in five domains: adaptive, gross motor, fine motor, language, and personal-social. Children were considered as having a developmental concern if their DQ in any specific domain was  $\leq 75$ .<sup>13,14</sup>

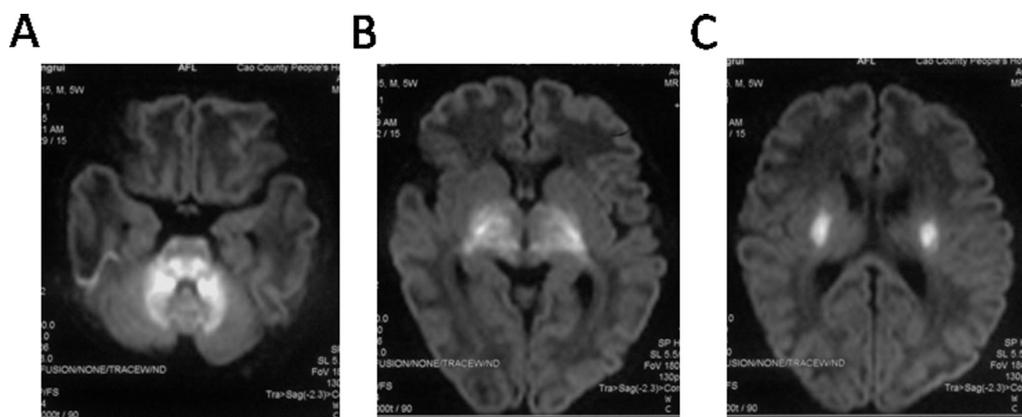
### 2.4. In silico analysis

Different software was used to predict significance of the mutations. Polyphen and Mutationtaster were used for missense mutation (<http://genetics.bwh.harvard.edu/pph/>, [www.mutationtaster.org/](http://www.mutationtaster.org/)).<sup>4</sup> Mutationtaster was used for deletion mutation.<sup>4</sup> Functional effects of point mutations were predicted with PredictProtein ([www.predictprotein.org](http://www.predictprotein.org)).<sup>15</sup> The secondary structure distributions of E1 $\beta$  were predicted with Phyre2 ([www.sbg.bio.ic.ac.uk/phyre2](http://www.sbg.bio.ic.ac.uk/phyre2)).<sup>16</sup>

## 3. Results

### 3.1. Clinical data and laboratory examinations

The patient was the first child of healthy non-consanguineous couple, born at 36 + 2 weeks by cesarean section, with a birth weight of 2700 g. There was no history of birth asphyxia. The boy had been breastfed. At the age of 40 days, he was admitted to hospital due to irritability and opisthotonus-like posture. He had no obvious dysmorphic features. Cardiovascular, respiratory and abdominal examination was normal. One day after admission, plasma amino acid analysis was performed by tandem mass spectrometry and revealed 825  $\mu\text{mol/L}$  leucine (normal range < 161  $\mu\text{mol/L}$ ), 289.42  $\mu\text{mol/L}$  isoleucine (normal range < 161  $\mu\text{mol/L}$ ) and 489.95  $\mu\text{mol/L}$  valine (normal range < 282  $\mu\text{mol/L}$ ). Furthermore, the levels of urine organic acid were measured with gas chromatography-mass spectrometry. The patient demonstrated elevated 2-ketoisocaproic acid, 2-keto-3-methylvaleric acid and 2-hydroxyisovaleric acid. Therefore the patient was diagnosed as MSUD. Magnetic resonance imaging (MRI) was performed on the third day of admission. Diffusion-weighted imaging (DWI) revealed restricted diffusion in bilateral cerebellar white matter, brainstem, thalami, globi pallidi, posterior limbs of internal capsules and corona radiata (Fig. 1), but brain edema was not observed.



**Figure 1** DWI showed hyperintense signal in bilateral dorsal brainstem, white matter of cerebellum (A), thalami, globi pallidi, posterior limbs of internal capsules (B) and corona radiata (C).

### 3.2. Genetic testing and in silico analysis

Two novel *BCKDHB* mutations (c.523 T > C (p.F175L) and c.478-25\_552del100) were identified. Direct sequencing analysis of the parents revealed that the mother was heterozygous for the c. 523 T > C and the father was heterozygous for the deletion mutation.

In order to assess the effect of the two mutations, in-silico analysis was performed. According to PolyPhen-2 and Mutationtaster, the missense mutation (c.523 T > C) was predicted to be “probably damaging” (HumVar score of 0.949) (Fig. 2) and “disease causing” (prob: 0.9999) independently. The deletion mutation was predicted to be “disease causing” (prob: 1) by Mutationtaster. Functional effects of the missense mutation were predicted with PredictProtein. The missense mutation was predicted to have a score of 49. The secondary structure distributions of E1 $\beta$  subunit were predicted with Phyre2 and are shown in Fig. 3. The deletion position of amino acids was between residue 160 and residue 184. The deletion mutation may have altered some secondary structure including the elimination of  $\alpha$ -helix (residues 162–170) and  $\beta$ -strand (residues 179–183).

### 3.3. Treatment and follow-up

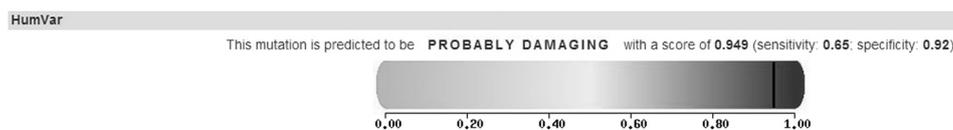
The patient was fed with BCAA-free formula and treated with L-carnitine during admission. The patient was non-responsive to thiamine. He tolerated the initial treatment well and had symptomatic improvement. Furthermore, the levels of plasma BCAAs and urine organic acids decreased significantly. Before discharge, the metabolic parameters reached the normal levels. The boy was discharged from the hospital 8 days later with advice to the parents to restrict dietary intake of BCAA. After discharge, plasma

BCAA levels of the patient were monitored strictly once per week. At the age of 10 months, the levels of leucine, isoleucine and valine were 246.59  $\mu\text{mol/L}$ , 101.78  $\mu\text{mol/L}$  and 177.33  $\mu\text{mol/L}$ , respectively. The levels of organic acids were within the normal range. Somatic growth of the patient was normal during 10-months’ follow up. His weight was 11 kg, his length was 75 cm, and his circumference was 46.5 cm. MRI was performed and showed improvement of the abnormalities on DWI (data not shown).

The developmental quotient (DQ) was evaluated using the Revised Gesell Developmental Evaluation.<sup>8</sup> The boy sat by himself but did not stand alone at 10 months (Fig. 4). The boy presented with mild hypotonia and had nearly normal DQ scores (gross motor 67, fine motor 76, language 76, adaptive 76 and personal-social 86).

### 4. Discussion

In this study, we describe the clinical presentation, biochemical features, gene mutations, treatment and follow-up of a case with MSUD. Definitive laboratory diagnosis is crucial to confirm MSUD since its clinical presentation is non-specific and can mimic common conditions such as infections and other inborn errors of metabolism.<sup>17</sup> Blood BCAAs analysis is the most convenient method, and urine organic acid analysis is helpful for the diagnosis and differential diagnosis of other organic acidurias. Here, the patient had typical findings of MSUD from plasma amino acids and urine organic acids analyses. In the classical MSUD, clinical onset usually occurs within the first week after birth. Patients with classic MSUD usually present with ketonuria, irritability, and poor feeding at the age of 2–3 days, and worsening encephalopathy at the age of 4–5 days. **If untreated, coma and even respiratory failure can occur at the age of 7–10 days.**<sup>7</sup> However, patients with



**Figure 2** The PolyPhen predicted the c.523 T > C (p.F175L) mutation may affect protein function with a score of 0.949.



**Figure 3** The secondary structure of BCKDH E1-beta was shown.

intermediate MSUD have markedly increased BCAA levels and neurological impairment (such as feeding problems, mental retardation and developmental delay), and they may present much later in life. In our study, the boy was admitted to hospital due to irritability and opisthotonus-like posture at the age of 40 days. Based on the clinical presentation and later onset, the patient might be classified into intermediate form. Severe leucinos, brain swelling, and death can occur if individuals with intermediate MSUD are subjected to sufficient catabolic stress. Therefore, the distinction between classic and intermediate type is not absolute. Furthermore, other modifying genes may affect clinical presentation of MUSD. For example, the kidney of the child may do a better job to clear acid or have may be a better buffer system.

Although the measurement of enzyme activity is important for MSUD diagnosis, we did not assay activity of BCKDH due to lack of technical capability. Instead, we investigated mutational defects in three genes associated with MSUD. The analysis of *BCKDHA*, *BCKDHB* and *DBT* genes allowed for the successful identification of the putative causal mutations in the patients with MSUD. In this study, two *BCKDHB* mutations, c.523 T > C (p.F175L) and c.478-25\_552del100, were identified in the patient. In order to identify the mutations, sequencing data were compared with an integrated set of variants ([www.hgmd.cf.ac.uk](http://www.hgmd.cf.ac.uk)) and ExAC database (<http://exac.broadinstitute.org>). We did not find the two mutations in Human Gene Mutation Database and ExAC database. Thus, the two mutations are novel. Furthermore, both mutations were predicted to be "disease causing" by Mutationtaster. Here, the effects of point mutations were predicted with PredictProtein. Each substitution for each position of a protein in a heatmap representation was shown independently. The c.523 T > C mutation was predicted to have a score of 49 ( $-50 < \text{score} < 50$ , weak signal for effect), which seemed to indicate small effect of the point mutation on function. We used Phyre2 to predict the secondary structure distribution. The deletion mutation maybe altered some secondary structure including the elimination of  $\alpha$ -helix (residues 160–168) and  $\beta$ -strand (residues 180–183). It seemed that the large deletion had high impact in protein function.

Neuroimaging should be performed to identify evidence of brain damage. DWI is a technique where diffusion of water molecules can be detected and is more sensitive than conventional MRI in detecting MSUD brain alterations.<sup>1</sup> Now it appears to be a valuable tool for early diagnosis and follow-up of metabolic disease in the neonates. In this case, the changes of neuroimaging were in the characteristic pattern of MSUD on DWI. Follow-up DWI may be of predictive value for the efficacy of treatment. At the age of 10 months, the follow-up MRI was performed and showed improvement of the abnormal signal.

Neurological sequelae are common in MSUD patients. The accumulation of branched-chain amino acids (especially leucine) and  $\alpha$ -ketoacid by products are considered to be the main neurotoxic metabolites. It has been postulated that metabolite accumulation in MSUD causes demyelination, inhibition of brain energy metabolism, induction of



**Figure 4** The boy with MSUD was able to sit unaided at the age of 10 months.

oxidative stress and apoptosis.<sup>18</sup> The aim of treatment is to protect the brain as early as possible from functional disturbances. To avoid brain damage, MSUD patients require lifelong dietary restriction and strict monitoring of the BCAAs levels to keep the branched-chain compounds in near-normal range.<sup>19,20</sup> The patient was fed with protein-restricted diet and treated with L-carnitine. L-carnitine has demonstrated antioxidant activity by reducing free radicals and by enhancing enzymic activity involved in the defense against reactive species.<sup>21,22</sup> Furthermore, L-carnitine has been reported to benefit patients with MSUD by preventing oxidative damage.<sup>20</sup> The boy responded well to treatment and showed clinical improvement. During the 10-month follow-up, the patient presented with mild hypotonia and had nearly normal DQ scores.

In view of clinical data of the patient, prompt diagnosis and treatment is essential to improve neurological outcomes and close monitoring of BCAA levels is crucial. Furthermore, we identified two novel mutations of the *BCKDHB* gene. The two novel mutations result in the clinical manifestation of MSUD, expanding the mutation spectrum of MSUD. Our results may reflect the heterogeneity of the pathogenic variants found in patients with MSUD. To study the mutation spectrums of disease-causing genes associated with MSUD in Chinese population, a large-scale investigation is necessary.

## Potential conflict of interest report

The authors indicated no potential conflict of interest.

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