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ORIGINAL ARTICLE

Two homozygous mutations in the exon 5 of BCKDHB gene that may cause the classic form of maple syrup urine disease

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Abstract Maple syrup urine disease (MSUD) is a rare autosomal recessive genetic disorder caused by defects in the catabolism of the branched-chain amino acids (BCAAs). Classic form of MSUD (CMSUD) is caused by mutations in BCKDHA, BCKDHB, DBT genes mostly. In this study, we analyzed the clinical and genetic characteristics of two patients with CMSUD. Two homozygous mutations, c.517G > T (p.Asp173Tyr) and c.503G > A (p.Arg168His), both in the exon 5 of BCKDHB were detected respectively. The novel mutation p.Asp173Tyr of patient A, inherited from his parents, is predicted to affect conformation of protein by computer analysis. The reported mutation p.Arg168His observed in patient B seemed to occur in a maternal uniparental disomy inheritance manner. Review of related literature revealed that most missense mutations in exon 5 of BCKDHB in homozygous genotype often result in CMSUD because of its incorrect conformation, and exon 5 of BCKDHB might be a susceptible region. Thus the novel homozygous mutation p.Asp173Tyr and the founder homozygous mutation p.Arg168His may be responsible for the clinical presentation of the two CMSUD patients, facilitating the future genetic counselling and prenatal diagnosis.

Keywords Maple syrup urine disease (MSUD) ·
Branched-chain amino acids (BCAAs) · BCKDHB gene

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Background

Maple syrup urine disease (MSUD, OMIM248600) is a rare autosomal recessive genetic disorder caused by defects in the catabolism of the branched-chain amino acids (BCAAs). The branched-chain α-keto acid dehydrogenase (BCKD) complex, a mitochondrial multienzyme complex, is comprised of three catalytic components: a branched-chain α-keto acid decarboxylase (E1), including two E1α and two E1β subunits, a dihydrolipoyl transacylase (E2), and a dihydrolipoamide dehydrogenase (E3), encoded by nuclear BCKDHA, BCKDHB, DBT and DLD genes respectively. Defective activity of the BCKD complex caused by mutations in these four genes will lead to elevated BCAAs, such as leucine, isoleucine and valine and an accumulation of branched-chain α-keto acids (BCKAs) in cells and body fluids (Nellis et al. 2003). Moreover, the E3 component is also shared by pyruvate and α-ketoglutarate dehydrogenase complexes, and patients with mutations in DLD may present a specific syndrome (dihydrolipoamide dehydrogenase deficiency) with congenital lactic acidosis (Couce et al. 2015; Grafakou et al. 2003).

Based on residual BCKD complex activity, clinical presentation and onset age, MSUD can be divided into five forms (Miryounesi et al. 2015): the classic form with less than 2% residual activity presents the most severe type and account for almost 75% cases. The intermediate form has 3–8% residual activity and the intermittent form exhibits 8–15% residual activity. The clinical presentation of these two forms varies considerably (Axler and Holmquist 2014; Guo et al. 2015). Thiamine-responsive form is considered to be sensitive to therapy based on thiamine. The last form refers to the MSUD with E3 deficiency.

Patients with classic phenotype develop symptoms between 4 and 9 days of age, which are characterized by fatal ketoacidosis, neurological impairments and mental retardation

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(Couce et al. 2015). The elevated levels of BCAAs and branched-chain α-keto acids in serum may confirm the diagnosis (Miryounesi et al. 2015). A timely treatment will prevent some patients from suffering developmental delay. For MSUD, patients require life-long dietary restriction (Li et al. 2015; Morton et al. 2002), and the early diagnosis and treatment are very important to reduce the severity of brain injury (Strauss et al. 2010).

BCKDHB, the gene encoding E1β (a 392-amino-acid protein), consists of 10 exons and is located on chromosome 6 [6q14.1]. Up to date, at least 84 mutations have been described according to the Human Gene Mutation Database (HGMD,www.hgmd.cf.ac.uk), most of which are missense mutations. Today, we report two newborns with classic form of MSUD caused by two homozygous mutations (p.Asp173 Tyr, p.Arg168His) in exon 5 of BCKDHB gene in two families, to demonstrate the potential correlation between phenotype and genotype of the disease, and describe a specific inheritance pedigree.

Clinical presentation and clinical course

Patient A Patient A in this study (a 2-month-old male at the time of the study) was the first child of two non-consanguineous healthy Chinese parents. He was admitted and treated in a hospital in Guangzhou, and presented with poor feeding, irregular breathing when the boy was 9 days old. Then plasma amino acid analysis showed the elevated BCAAs, with leucine at 1773.77 μmol/L (normal range 55–167 μmol/L), so the classic form of MSUD was diagnosed. He received assisted respiration and a low-protein diet. Gradually he recovered and was released from the hospital when he was 1-month old. However, 15 days later, he showed a cough, difficult breathing and rash on the skin of body, without fever and convulsion, so he was readmitted in our hospital. During the period, liver function, renal function, blood lactic acid and blood ammonia were tested and abnormalities were not found, yet plasma amino acid analysis was performed again and showed 1075.2 μmol/L leucine (normal range 4.0–197.0 μmol/L), 413 μmol/L valine (normal range 99–333.0 μmol/L) and 8.9 μmol/L isoleucine (normal range 24–118.00 μmol/L). As for the rash in his body, probably as a result of lacking the essential amino acids or some minerals, he was advised to take 1/3 infant formula (Enfamil® A+ Asia) and 2/3 dietary leucine restriction, and finally he had less cough with rash disappeared. Materials of skull CT and electroencephalography were unavailable, for he left the hospital soon after 2 days of admission. However, his motor development and cognitive function at 2-month old are nearly normal.

Patient B Patient B was an 11-day-old boy, an ethnic Han Chinese. His parents were not consanguineous, and he

appeared a normal birth weight (2.6 kg). He presented with poor feeding and weight loss 6 days later (only 1.86 kg). In a local primary hospital he was considered a suspected MSUD case and transferred to our hospital for further treatment. On the first day of hospitalization, he developed seizures with abnormal electroencephalography, accompanied by a maple syrup odor from the urine. Lab tests revealed coagulation disturbance, hyperammonemia and acidosis. Elevated α-ketoisocaproic acid, α-ketoisovaleric acid, α-keto-β-methylvaleric acid and lactic acid appeared in urine by GC-MS analysis. The elevated BCAAs were detected in plasma amino acid analysis, with leucine at 3729 μmol/L (normal range 4–197 μmol/L), isoleucine at 790.4 μmol/L (normal range 24–118 μmol/L), and valine at 604 μmol/L (normal range 99–333 μmol/L). He was diagnosed with the classic form of MSUD, and was administered Vitamin B1、carnitine and a low-protein diet. Ten days later the levels of leucine in plasma had dropped to 2247 μmol/L. Nevertheless, he exhibited a poor response to the therapy, and showed irregular breathing due to central respiratory failure. Skull CT scanning demonstrated enormous flakes of symmetrical low density areas in white matter in the cerebral hemisphere, basal ganglia, bilateral thalami, brainstem and cerebellum (Fig. 1). The patient died 23 days after birth.

Methods

Genomic DNA of these two patients and their parents was extracted from peripheral blood leukocytes according to manufacturer's protocol (QIAGEN). Polymerase-chain reaction (PCR) was used to amplify all coding exons and flanking exon/intron boundaries of the BCKDHA, BCKDHB and DBT genes. PCR products were detected on agarose gel and directly sequenced. The sequences obtained were analyzed by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the cDNA sequences for BCKDHA, BCKDHB and DBT in GenBank. Mutations were reconfirmed by PCR amplification and subsequent sequencing by using both the forward and reverse primers. Genetic analysis was approved by the institutional review board of Guangzhou Women and Children' Hospital with informed consent of the parents.

The SIFT/PROVEAN and Polyphen2 program were utilized to predict the effects of missense substitutions on protein function. In order to verify the conversation of species, multiple sequence alignment were performed by using ClustalX (http://www.clustal.org/clustal2/). Crystal structures (1X7Y) were retrieved from Protein Data Bank.

Fig. 1 Skull CT scanning and Chest X-ray of the patient B. **a**, **b** Skull CT scanning demonstrated enormous flakes of symmetrical low density areas in the white matter of the cerebral hemisphere, basal ganglia, bilateral thalami, brainstem and cerebellum at the 22th day after birth. **c** At the 12th day after birth, chest X-ray indicated slightly thickened double lung texture. **d** At the 22th day after birth, chest X-ray indicated a patch of hazy shadow in double lung caused by severe neurological impairment
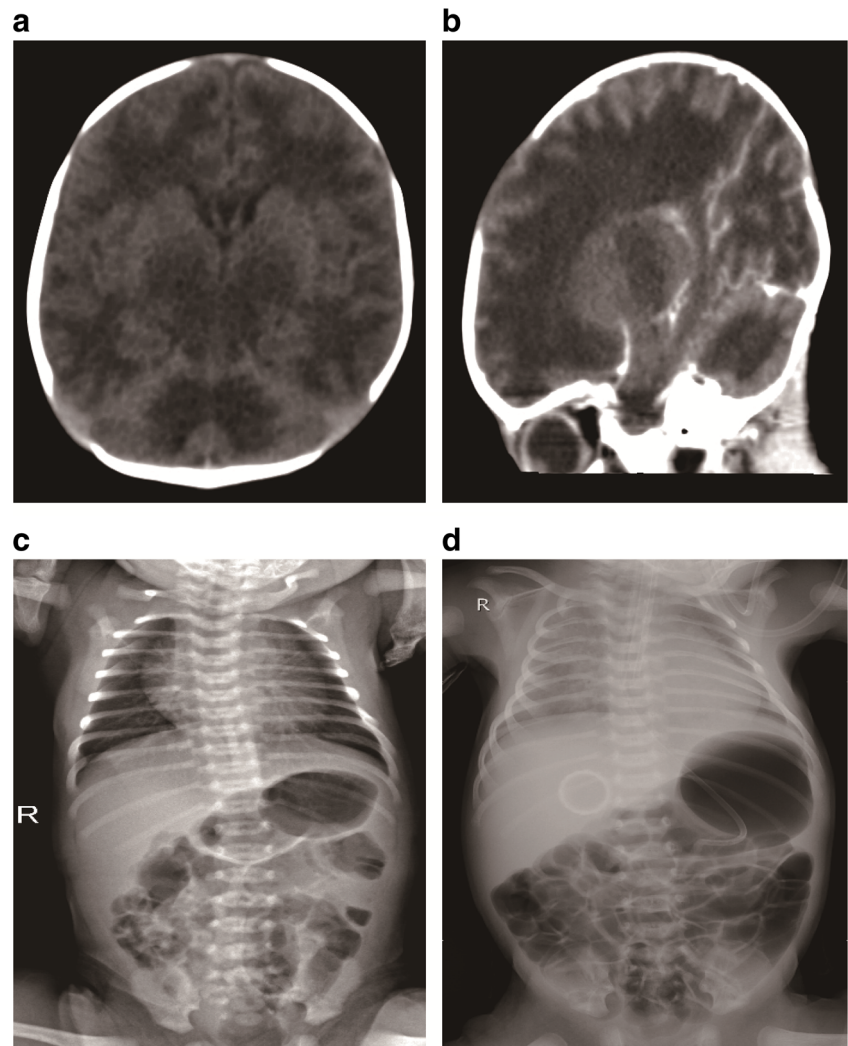
Results

In patient A, no pathogenic mutations were detected in the BCKDHA or DBT Gene, a novel missense mutation c.517G > T (p.Asp173Tyr) in the BCKDHB gene in a homozygous state was identified. Direct sequencing analysis of the gene of his parents showed the same missense mutation in exon 5 in a heterozygous state, demonstrating that the novel mutation p.Asp173Tyr was parentally inherited (Fig. 2). The mutation was predicted to be probably damaging with a score of 0.995 by Polyphen2 software. The SIFT/PROVEAN software was also used to predict the significance of the mutation, similarly, showing that the mutation was deleterious or damaging. ClustalX analysis demonstrated that this amino acid site was conservative among different species. Computer simulation (Pymol software) based on amino acid sequence showed that the mutation changed the structure of the protein (Fig. 3).

In patient B, the reported mutation c.503G > A in exon 5 resulted in an amino acid substitution of arginine for histidine at position 168 in a homozygous state. His mother was found

to be a carrier of the pathogenic mutation, however the same mutation was not detected in exon 5 of his father (Fig. 2). Sixteen short tandem repeat (STR) genetic loci on the chromosome 13, 18, 21 of patient B and his parents were amplified respectively, and PCR products were analyzed by capillary electrophoresis and GeneMapper software. The result showed all STR genetic loci in this family could be explained by Mendelian principles, so their parenthood was confirmed (Fig. 4). Homozygous state was validated by exome sequencing, excluding the possibility of gene deletion (data not shown).

Discussion

In our study, these two patients with mutations in exon 5 of BCKDHB gene both presented the classic form of maple syrup urine disease with varying degree of clinical manifestations. Patient A, who showed feeding difficulties and irregular breathing at day 9, was treated by a restricted diet and
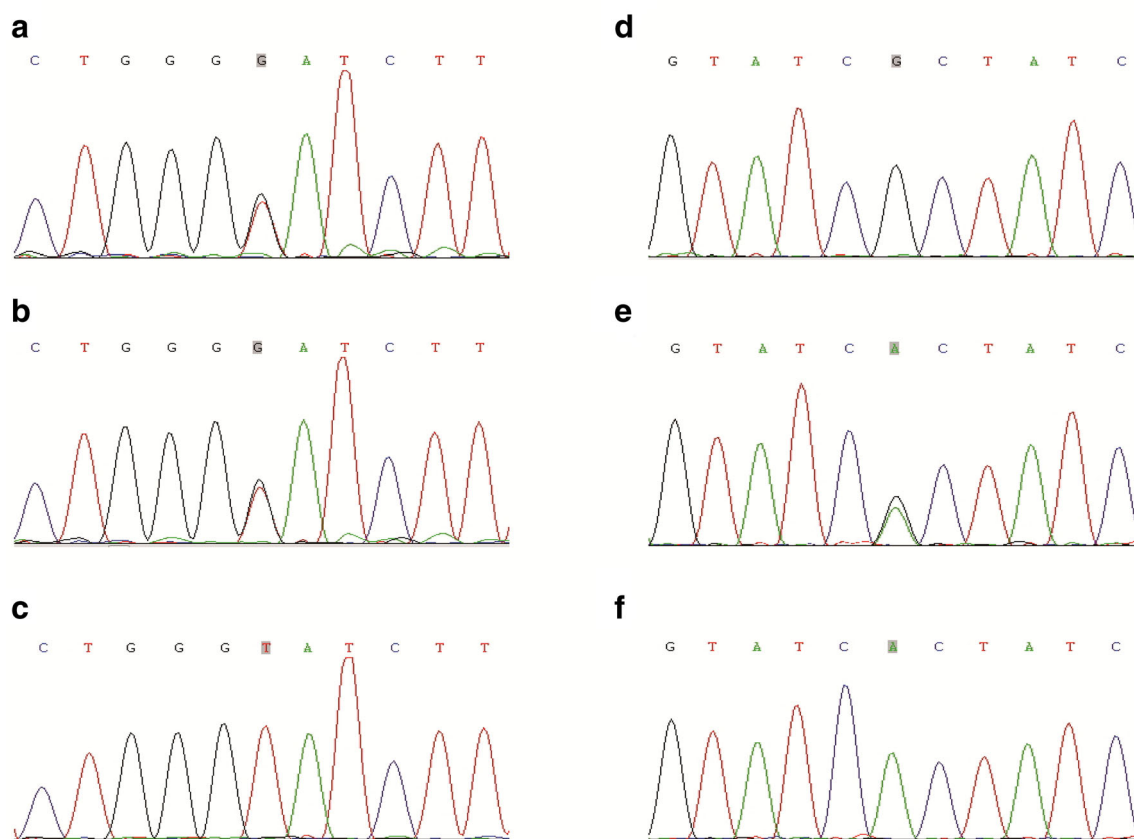
Fig. 2 Genetic mutations of the patient with maple syrup urine disease in our study. **a**, **b**, **c** Two heterozygotes parents (G/T) (**a** and **b**) with a mutation at the same site and their homozygote (T/T) (**c**) child. **d**, **e**, **f** A homozygote father (G/G), a heterozygote mother (G/A) and their homozygote child with a genetic mutation (A/A)

symptomatic approach, and recovered well. Though he coughed repeatedly, the presentation was mild. Referring to the rash on his body, simplistic diet might be the cause. Despite BCAA-free formula, supplementation with isoleucine and valine should be included (Couce et al. 2015; Li et al. 2015). When given dietary supplements, patient A didn't suffer from rash again. The patient didn't show any neurological signs such as seizures, convulsions, indicating less neurological impairments. Nevertheless patient

B exhibited much more severe manifestations, including convulsion, irregular breathing, and eventually died. The initial level of serum leucine in patient B was much higher than that in patient A, and the accumulation of BCAAs resulted in acute and chronic brain dysfunction in patient B (Couce et al. 2015; Hou and Hwang 2014). Although both homozygous mutations were detected in exon 5 of BCKDHB, the reported mutation c.503G > A had a larger impact on the BCKDC activity.

Fig. 3 Spatial correlations of the residue p.Asp173 in β subunit of 1X7Y. **a** and **b** represent p.Asp173 and mutated p.Asp173Tyr, respectively. *Red arrow* is pointed to the 173th amino acid of the protein, and red numbers are the length of the hydrogen bond
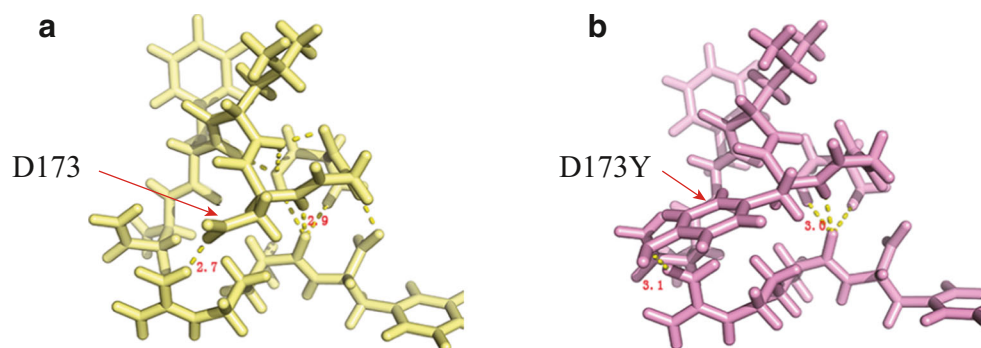
Fig. 4 STR map illustrates the parenthood of the family. Each STR appears as a peak in a position that corresponds to its repeat number. There are a pair of peaks for every STR either inherited from mother or from the father. **a**, **b**, **c** represent the father, mother and child, respectively. This relationship is consistent with Mendelian inheritance
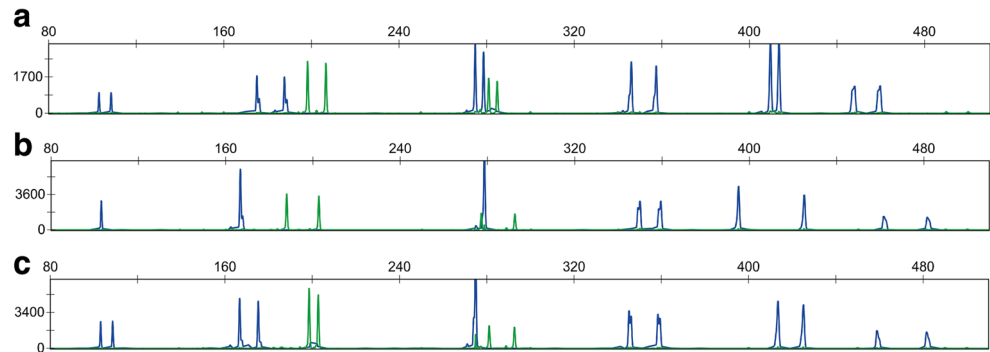
The missense mutation c.517G > T (p.Asp173Tyr) in patient A has not been reported before. Due to the site conservation among different species, the p.Asp173Tyr mutation at the α-β interface is predicted to be probably damaging and deleterious in that a tyrosine with a phenyl ring may hamper subunit assembly. p.Asp173Tyr was less likely to be considered a gene polymorphism, for the same mutation was not found in Exome Aggregation Consortium (EXAC, http://exac.broadinstitute.org/) or The International Genome Sample Resource (IGSR, http://www.internationalgenome.org/1000-genomes-browsers). Although the patient with the mutation presented the classic phenotype, and received intubation and assisted respiration because of neurological impairment in his first hospitalization, he was diagnosed and treated timely and recovered well with a good prognosis. Maybe this is because the brain lesion is fully reversible during the transient neonatal period provided that earlier diagnosis and treatment are obtained (Couce et al. 2015). Of course observations for a long time to assess the psychomotor development are also needed.

The founder mutation c.503G > A in patient B results in Arg168His amino acid substitution at the α-β' subunit interface. This nucleotide change has been previously reported (Couce et al. 2015; Rodriguez-Pombo et al. 2006). More severe clinical phenotypes, such as classic phenotype with neurological symptoms in two Spanish patients, were also associated with the mutation. Luckily both of them were recovered with relatively favorable prognoses, one with IQ > 85 and the other had developmental delay. In our study, the patient with the same mutation also manifested the severe classic form, which was consistent with the literatures. However, the patient was diagnosed on the day 11, the neurological impairment had formed. Worse, the high levels of BCAAs (>2 000umol/L) lasted for 11 days during the whole admission. Accumulation of leucine and its metabolite, α-ketoisocaproic acid, in plasm are involved in the presentation of neurological symptoms (Couce et al. 2015; Funchal et al. 2007). Neonatal encephalopathy may lead to a four times higher risk of global functional impairment (Muelly et al. 2013). According to the skull

CT scanning, the patient had severe neurological impairment, which resulted in a central respiratory failure. The patient died on the day after his parents abandoned further treatment. In general, prognoses varied from different onsets, treatments or individual factors, although they possessed the same pathogenic mutation.

Up to date, twenty one pathogenic mutations in exon 5 of BCKDHB have been reported in HGMD database. By reviewing related literatures, we analyzed all the clinical manifestations related to reported mutations in exon 5. Table 1 lists MSUD patients with mutations in exon 5 of the BCKDHB gene. In these reports all patients with founder mutations in homozygous state showed classic style of MSUD, with various neurological complications such as seizures, hypotonia and lethargy (Chuang et al. 2004; Edelmann et al. 2001; Gorzelany et al. 2009; Gupta et al. 2015; Miryounesi et al. 2015; Rodriguez-Pombo et al. 2006), and many of them died at last. Among those 17 patients with pathogenic mutations in exon 5 in only one allele, nine presented classic form, three exhibited intermediate style, three demonstrated mild variant, and there are no data for the remaining two. Two molecular studies revealed that BCKDHA and BCKDHB could be the major genes involved in MSUD in Indian populations (Bashyam et al. 2012; Narayanan et al. 2013), so was in Chinese and Spain populations (Rodriguez-Pombo et al. 2006; Yang et al. 2012). Missense mutations in exon 5 account for 25% of all mutations of BCKDHB according to HGMD. Amino acids in exon 5 of BCKDHB often residue at homodimers interface of β-β' subunits and (or) at interface of α-β which are critical for maintaining the correct spatial interactions of both β-β' and α-β subunits (Bashyam et al. 2012). Missense mutations in this region would impair the precise conformation of heterotetrameric structure, subsequently hamper subunit assembly and inactivate the enzymatic function, and finally lead to the severe classic type of MSUD (McConnell et al. 1997). Thus Exon 5 in BCKDHB may be a susceptible exon and mutations in exon 5 may have a significant impact on residual activity. In our study, homozygous mutations c.517G > T and c.503G > A were also detected in exon 5 of

Table 1 MSUD patients with mutations in exon 5 of BCKDHB gene

Pathogenic mutations	Gender	Age of onset	Clinical features	Clinical phenotype	Homozygote/Heterozygote	Prognosis	Reference
Asp173Tyr	F	9d	Poor feeding, irregular breathing	Classic	Homozygote	Well, need further observation	This study
Arg168His	F	6d	Poor feeding, convulsion, irregular breathing	Classic	Homozygote	Died at 23 days	This study
Gly172Trp	M	7d	Poor feeding, coma, irritability	Classic	Compound heterozygote (Lys116fs)	IQ89 (8 years)	Couce et al. 2015
Arg168His	F	10d	Poor feeding, irritability	Classic	Compound heterozygote (Arg324stop)	IQ > 85	Couce et al. 2015
Glu163X(3[a])	NA	NP	NA	Classic	Homozygote	died	Rodriguez-Pombo et al. 2006
Arg168His	NA	NP	NA	Classic	Homozygote	Developmental delay	Rodriguez-Pombo et al. 2006
Gly172Trp	NA	NP	NA	Classic	heterozygote (348delA)	Loss to follow up	Rodriguez-Pombo et al., 2006
Arg170His	M	7d	Poor feeding, seizures, hypotonia and hypertonia	Classic	Compound heterozygote (Gln346Arg)	Died at 10 days	Wang et al. 2012
Val161Gly	F	9d	(−)	Not classified	Compound heterozygote (Arg170Cys)	Well	Li et al. 2015
Pro185Leu	M	NP	Neonatal encephalopathy, seizure	Classic	Homozygote	Died at 10 months	Gupta et al. 2015
Arg183Pro	F	6 m	Developmental delay	Intermediate	Compound heterozygote (Thr332Ala)	Developmental delay	Gupta et al. 2015
Pro181Leu	NA	7 m	NA	Mild variant	Compound heterozygote (Gly135Arg)	NA	Narayanan et al. 2013
Pro200Ala	NA	NS	(−)	Mild variant	Compound heterozygote (Arg111X)	NA	Flaschker et al. 2007
Arg168Cys	NA	NA	(−)	Mild variant	Compound heterozygote (Val283Ala)	NA	Simon et al. 2006
Asn176Tyr	NA	NA	NA	NA	Compound heterozygote (R274X)	NA	McConnell et al. 1997
Arg183Gln	F	5d	NA	Classic	Compound heterozygote (Thr322Ala)	NA	Bashyam et al. 2012
Arg170Cys	F	2 m	Poor feeding, seizures, hypotonia, lethargy	Classic	Homozygote	Died at 6 months	Miryounesi et al. 2015
Arg183Trp	NA	10d	NA	Classic	Homozygote	NA	Gorzelany et al. 2009
Cys188Stop	NA	5d	NA	Classic	Homozygote	NA	Gorzelany et al. 2009
Arg183Pro(4[b])	NA	NA	NA	Classic	Homozygote	NA	Edelmann et al. 2001
Arg183Pro(2[c])	NA	NA	NA	Classic /intermediate	Compound heterozygote (NA)	NA	Edelmann et al. 2001
Gln198Leu	NA	10d	(−)	Intermediate		Well	Yang et al. 2012

Table 1 (continued)

Pathogenic mutations	Gender	Age of onset	Clinical features	Clinical phenotype	Homozygote/ Heterozygote	Prognosis	Reference
					Compound heterozygote (C349Y)		
Asn176Tyr	F	11d	Coma, ataxia	Classic	Compound heterozygote (R324X)	NA	Nellis et al. 2003
Val189Asp	M	10d	NA	Classic	Compound heterozygote (A137V)	IQ 96	Nellis et al. 2003
His206Tyr	NA	NA	NA	Classic	Homozygote	NA	Chuang et al. 2004
Leu194Phe	M	7d	Poor feeding, seizures	Classic	Compound heterozygote (Ser199Arg)	Died at 16 days	Shen et al. 2015

NA not available, *NP* neonatal period, *NS* neonatal screening, *IQ* intelligence quotient

[a] 3 cases with same mutation

[b] 4 cases with same mutation

[c] 2 cases with same mutation

BCKDHB of two infants with classic phenotype, respectively, and these were consistent with our hypothesis.

The parents of patient A were both proved to be carriers of the mutation p.Asp173Try by sequencing the exon 5 of BCKDHB. As for patient B, the more complicated scenario is that the known mutation in patient B seemed to be inherited only from his mother, as the same mutation was not detected in his father. Linkage analysis of common STR has confirmed the parentage. Exome sequencing showed that no deletions or other pathogenic mutations exist in patient B. That suggested maternal uniparental disomy might be involved. Nondisjunction in maternal meiosis resulted in an oocyte with two copies of the pathogenic mutant allele. The oocyte, fertilized by a sperm that did not carry a paternal chromosome 6 or subsequent mitotic loss of the paternal chromosome 6, can lead to patient B possession of two mutant BCKDHB alleles on two maternal chromosomes. Of course, a novel mutation happened in exactly the same position in the allele derived from his father may also contribute to such an outcome, but the chance is quite slim.

In conclusion, in this study we analyzed the clinical and genetic characteristics of two MSUD patients with homozygous mutations in exon 5 of the BCKDHB gene. Missense mutations in exon 5 of BCKDHB in homozygous genotype often result in classic form of MSUD owing to its incorrect conformation. Exon 5 of BCKDHB is a susceptible region, and the novel mutation c.517G > T (p.Asp173Tyr) may be responsible for the disease. In specific inheritance pedigree, further analysis and research are needed to facilitate genetic counseling and prenatal diagnosis.

Compliance with ethical standards

Conflict of interest statement The authors declared that they have no conflicts of interest to this work.

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