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**Diagnosis of an intermediate case of maple syrup urine disease: A case report**

Lin YT *et al.* An intermediate case of MSUD

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## **Abstract**

### BACKGROUND

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Maple syrup urine disease (MSUD) is an autosomal recessive genetic disorder caused by defects in the catabolism of the branched-chain amino acids (BCAAs). However, the clinical and metabolic screening is limited in identifying all MSUD patients, especially those patients with mild phenotypes or are asymptomatic. This study aims to share the diagnostic experience of an intermediate MSUD case who was missed by metabolic profiling but identified by genetic analysis.

### CASE SUMMARY

This study reports the diagnostic process of a boy with intermediate MSUD. The proband presented with psychomotor retardation and cerebral lesions on magnetic resonance imaging scans at 8 mo of age. Preliminary clinical and metabolic profiling did not support a specific disease. However, whole exome sequencing and subsequent Sanger sequencing at 1 year and 7 mo of age identified bi-allelic pathogenic variants of the *BCKDHB* gene, confirming the proband as having MSUD with non-classic mild phenotypes. His clinical and laboratory data were retrospectively analyzed. According to his disease course, he was classified into intermediate form of MSUD. His management was then changed to

BCAAs restriction and metabolic monitoring conforming to MSUD. In addition, genetic counseling and prenatal diagnosis were provided to his parents.

## CONCLUSION

Our work provides diagnostic experience of an intermediate MSUD case, suggests that a genetic analysis is important for ambiguous cases, and alerts clinicians to avoid missing of patients with non-classic mild phenotypes of MSUD.

**Key Words:** <sup>8</sup>Maple syrup urine disease; *BCKDHB* gene; Branched-chain amino acids; Metabolic profiling; Genetic analysis; Case report

Lin YT, Cai <sup>6</sup>YN, Ting TH, Liu L, Zeng CH, Su <sup>2</sup>L, Peng MZ, Li XZ. Diagnosis of an intermediate case of maple syrup urine disease: <sup>2</sup>A case report. *World J Clin Cases* 2022; In press

**Core Tip:** <sup>1</sup>Clinical and metabolic screening is limited in identifying all patients with maple syrup urine disease (MSUD), especially those patients with mild phenotypes or are asymptomatic. Here we present the diagnostic process of an intermediate MSUD case with a mild phenotype, who was missed by metabolic profiling but identified by genetic analysis. This case suggests that genetic testing could contribute to the identification of non-classic mild MSUD cases, and alerts clinicians to avoid missing of these patients.

## INTRODUCTION

Maple syrup urine disease (MSUD), named for a maple syrup odor in urine, is a rare inborn error of metabolism, with an estimated prevalence of 1/185000 live births<sup>[1]</sup>. Also known as branched-chain  $\alpha$ -ketoacid dehydrogenase (BCKD) deficiency, MSUD is caused by defects of the mitochondrial BCKD complex which leads to disruption in the catabolism of the branched-chain amino acids (BCAAs), namely leucine, isoleucine, and valine. To date, five different genes, *BCKDHA*, *BCKDHB*, *DBT*, *DLD*, and *PPM1K*, have been identified as genetic causes of MSUD, all of them having an autosomal recessive inheritance pattern<sup>[2-6]</sup>.

MSUD is characterized by clinical manifestations of neurological impairments, feeding problems and metabolic decompensation, and metabolic profile of elevated BCAAs in the plasma and elevated branched-chain ketoacids (BCKAs) in the urine. According to the defective component of the BCKD complex and the severity of the phenotypes, MSUD can be divided into five forms, namely classic, intermediate, intermittent, thiamine-responsive, and E3-deficient<sup>[1,7]</sup>.

Generally, MSUD is diagnosed by the presence of clinical features accompanied by characteristic plasma and urine metabolic profiles, and further confirmed by a genetic analysis. The management is comprised of BCAAs restriction and metabolic monitoring, which typically yields good outcomes. Moreover, domino liver transplantation provides an effective strategy to achieve a normal dietary intake<sup>[1,8-10]</sup>.

Early diagnosis and treatment is important for MSUD as acute metabolic crisis is life-threatening. However, the clinical and metabolic screening is limited in identifying all MSUD patients. Those mild cases under the BCAAs criteria to be considered presumptive positive for MSUD are probably missed, as well as intermittent patients with normal development and BCAAs levels when asymptomatic<sup>[11-14]</sup>. Thus, a supplementary genetic testing could contribute to the identification of ambiguous cases. To share the diagnostic experience and alert clinicians to avoid missing of diagnosis, here we present an intermediate case of MSUD who was missed by metabolic profiling but identified by

genetic analysis. Genetic counseling and prenatal diagnosis were further provided to the family.

## **CASE PRESENTATION**

### ***Chief complaints***

The proband is a boy aged 1 year and 7 mo with psychomotor retardation.

### ***Imaging examinations***

He had brain magnetic resonance imaging (MRI) performed by another hospital at 8 mo of age (data not shown) and our hospital at 1 year and 3 mo of age and 1 year and 7 mo of age (Figure 1C), showing abnormal signal in the right frontal lobe, and increased T2/FLAIR signals symmetrically in the basal ganglia and trigone of lateral ventricles.

### ***Laboratory examinations***

**Routine biochemical test:** Routine biochemical tests at 8 mo of age showed normal liver and renal function, blood glucose, blood ammonia, blood gas, and blood electrolyte (data not shown).

**Enzymatic assay:** The activities of sphingolipidoses-relevant enzymes at 8 mo of age were normal (data not shown).

**Metabolic analysis:** Quantitative plasma amino acid profiling by high-performance liquid chromatography and semi-quantitative urine organic acid analysis by gas chromatography-mass spectrometry were performed as described previously<sup>[16,17]</sup>.

Plasma amino acid profiling at 8 mo of age showed elevations of leucine, isoleucine, valine, and leucine/alanine ratio (Table 2), which met the specific amino acid spectrum of MSUD<sup>[11]</sup>. However, no maple syrup odor was noticed in his urine. Urine organic acid analysis revealed a small amount of 2-OH-isovaleric acid (Figure 2B).

To exclude the impact of dietary factors and further define if this boy had MSUD, he had repeated sampling and analysis of plasma amino acids and urine organic acids. On the second examination at 1 year and 1 mo of age, the concentrations of plasma BCAAs turned into normal (Table 2), while the peak of urine 2-OH-isovaleric acid became lower (Figure 2C), which did not support the possible diagnosis of MSUD. The proband was then categorized as unexplained psychomotor retardation.

**Genetic analysis:** gDNA was extracted from whole blood samples using DNeasy Blood & Tissue Kit (QIAGEN). Whole exome sequencing (WES) was performed as described previously<sup>[18]</sup>. For Sanger sequencing, the exon sequences together with adjoining intron boundaries of the *BCKDHB* gene (NG\_009775.2, NM\_183050.3) were amplified and sequenced with an ABI 3730xl DNA Analyzer. The SNP databases were employed to exclude the polymorphic alleles, while HGMD (Professional 2022.3) and ClinVar were referenced for the confirmation of known pathogenic variants.

To identify the underlying genetic cause, WES of the proband-parents trio was performed when the proband was at age of 1 year and 7 mo. To our surprise, a compound heterozygous c.584A>G(p.Tyr195Cys)/c.818C>T(p.Thr273Ile) variant of the *BCKDHB* gene was identified in the proband, one of which was transmitted from the unaffected parents respectively. Both of the identified *BCKDHB* variants were known to cause MSUD<sup>[13,19]</sup>. Sanger sequencing subsequently confirmed these results (Figure 3).

### *Physical examination*

The proband's weight and height were normal at presentation (Table 1)<sup>[15]</sup>. He didn't show facial dysmorphism. He had mild hypotonia without any pathological reflex. No obvious abnormalities were shown in his skeleton and viscera.

### *Personal and family history*

The family was of southern Chinese and Han ethnicity. The proband was the first child of the healthy parents (Figure 1A). He was delivered naturally at term with no perinatal



issues. The pregnancy was normal and the neonatal period was uneventful. <sup>13</sup> His parents were non-consanguineous, and there was no family history of psychomotor retardation or metabolic diseases. Spontaneous abortion of the second child of the parents occurred at 2 mo of pregnancy (Figure 1A).

## <sup>2</sup> *History of past illness*

He had no past admission or known medical problems. His development was normal in the first three months after birth.

## *History of present illness*

The proband presented to our clinic with psychomotor retardation at 8 mo of age as he was unable to sit. He achieved head control at 4 mo of age. Subsequently he began to sit at 11 mo of age, and <sup>5</sup> was able to stand and <sup>3</sup> walk independently at 1 year and 5 mo of age, but had a tendency to fall. He started babbling at 1 year and 5 mo of age, and <sup>3</sup> was unable to say a word or recognize his parents at 1 year and 7 mo of age.

## *Retrospective analysis*

The proband's semi-quantitative urine organic acid results were re-analyzed. In addition to a small amount of 2-OH-isovaleric acid, very small amounts of 3-OH-isovaleric <sup>14</sup> acid, 2-OH-isocaproic acid, 2-keto-isocaproic acid, and 2-keto-3-methylvaleric acid were noted (Figure 2B and C). These small fluctuations were missed previously. Thus, a characteristic urine organic acid spectrum of MSUD was concluded, supporting the molecular findings.

After repeated questioning, the parents recalled that the proband once vomited after eating meat and he tended to refuse eating meat since then, but could tolerate milk. There was no severe acute illness that required hospitalization. They also concealed the medical history that the proband was supplemented with trivitamins B tablets (containing vitamin B<sub>1</sub>, B<sub>6</sub>, and B<sub>12</sub>) at another hospital between the first two analyses of plasma amino acids and urine organic acids, which directly interfered with the levels of BCAAs.

### **FINAL DIAGNOSIS**

Based on the clinical phenotype, metabolic spectrum and molecular findings, the proband was confirmed to have MSUD with mild phenotypes at 1 year and 7 mo of age. According to his disease course, he was classified as intermediate form of MSUD. Moreover, he was responsive to thiamine (vitamin B<sub>1</sub>) as trivitamins B tablets supplement changed his plasma BCAAs from mild elevation into normal level.

### **TREATMENT**

The proband's management was then modified to BCAAs restriction and thiamine supplementation in conjunction with metabolic monitoring of plasma BCAAs and urine BCKAs.

### **OUTCOME AND FOLLOW-UP**

During the follow-up until 6 years and 2 mo of age, the proband's height and weight were persistently normal (Table 1). He could walk and run stably but slowly with mild hypotonia. His cognition and language were delayed for about 1 year comparing with healthy peers, but his parents refused an intelligence test. No seizure was observed. Moreover, there was still no maple syrup odor in his urine. His plasma BCAAs and urine BCKAs levels were decreased in comparison with the first examination before treatment, but fluctuated (Table 2 and Figure 2). His brain MRI at 3 years and 5 mo of age and 5 years of age showed a substantial reduction of the cerebral lesions (Figure 1C).

As the proband was diagnosed with MSUD and both of the parents carried a pathogenic heterozygous variant of the *BCKDHB* gene, a subsequent child of the parents has a recurrence risk of 25% to have MSUD. Prenatal diagnosis is offered to the parents to provide early diagnosis, which allows optimal management early at birth if the fetus is affected with MSUD. Preimplantation diagnosis is an alternative strategy to block the transmission of pathogenic variants. Moreover, members of the parental families can be offered screening genetic testing to identify potential carriers.



Prenatal diagnosis was provided for the mother's third pregnancy (Figure 1B). The chorionic villi biopsy tissues were obtained at 13 wk of gestational age. Sanger sequencing showed that the fetus carried the c.584A>G(p.Tyr195Cys) heterozygous variant transmitted from the father, while the c.818C>T(p.Thr273Ile) variant of the mother was not delivered to her (Figure 3). Thus, the fetus was defined as a carrier. The postnatal genetic testing confirmed this result (Figure 3). Her liver and renal function, and levels of plasma amino acids and urine organic acids were normal at 2 mo of age (data not shown). On follow-up until 3 year and 11 mo of age, she remained healthy with normal mental and motor development.

In addition, spontaneous abortion of the fourth child of the parents occurred at 11 wk of pregnancy before the prenatal diagnosis appointment (Figure 1B). Unfortunately, abortus samples were not retained for a further genetic testing.

## **DISCUSSION**

In MSUD, the classic form with the most severe phenotype presenting early in the first week of life accounts for most cases, while about 20% of cases with non-specific symptoms and varying clinical severity are classified into non-classic variant forms, including intermediate, intermittent, thiamine-responsive, and E3-deficient<sup>[7,13]</sup>. The classic cases with significantly elevated plasma BCAAs and urine BCKAs are easy to be detected by specific metabolic screening, while non-classic variant cases might be missed sometimes, especially when they are asymptomatic or have normal plasma BCAAs and urine BCKAs<sup>[11-14,20]</sup>.

In this work, we present the diagnostic process of an intermediate MSUD patient with a mild phenotype, who was missed by preliminary metabolic profiling but was later identified by genetic testing.

In retrospect, the proband could have been diagnosed during his first visit at 8 mo of age, because the first plasma amino acid profiling showed specific spectrum of MSUD. However, due to the elevation of BCAAs was not as significantly high as in the classic

form<sup>[20]</sup>, a verification analysis was required to exclude the impact of dietary factors. The subsequent verification analysis did not support the diagnosis of MSUD.

At that time, the parents did not provide adequate information for our inquiry as they were both young and inexperienced parents. They were also not well educated. They thought the history of meat refusal was not important as they believed it was usual and normal for young children to have dietary preferences. They also did not report the use of vitamin B supplement initially as they thought vitamins were routinely used for general health care of infants and children.

In China, trivitamins B tablets, levocarnitine (L-carnitine), and coenzyme Q10 tablets with minimal side-effects are often prescribed to children with psychomotor retardation. These supplements are perceived by parents to improve the psychomotor retardation to some extent. However, these supplements may interfere with the results of biochemical testing for metabolic diseases and hence hampers the prompt diagnosis of a specific metabolic disease.

Moreover, the urine organic acid analysis is semi-quantitative in our laboratory, which limits the detection sensitivity and efficiency. Very low peaks on the chromatograms were missed sometimes. In contrast, a quantitative urine organic acid analysis is more sensitive to improve diagnostic efficiency and accuracy.

The above-mentioned reasons led to the missing of MSUD diagnosis in this case based on laboratory metabolic indicators. Fortunately, the case was identified by WES although delayed. As the proband's unexplained psychomotor retardation was not a specific clinical feature of a metabolic disorder, it is difficult to determine a specific disease-causing gene to look for in genetic testing. Since traditional Sanger sequencing targeting a specific gene was unavailable, WES, one of next-generation sequencing (NGS) techniques, was employed as an alternative. Currently, NGS has been widely used in molecular diagnostic testing, especially in unexplained disorders, because it enables screening for a large amount of candidate genes rapidly in one test<sup>[21,22]</sup>.

In fact, the diagnosis of MSUD in the proband could be confirmed much earlier if we implemented genetic testing rather than re-examination of metabolites, when he was

suspected to suffer from MSUD. Either Sanger sequencing or targeted NGS involving the five causative genes of MSUD could have confirmed his diagnosis earlier. An earlier definitive diagnosis of MSUD would be beneficial by enabling genetic counseling and prenatal diagnosis for the family.

Generally, a metabolic disease is diagnosed by a characteristic metabolic profile followed by a confirmatory genetic analysis. Early detection and intervention is important for favourable prognosis. However, for some cases with mild non-classic or normal phenotypes, metabolic profiling may fail to detect the disease, and they could only be identified when a metabolic crisis episode occurs, which is usually life-threatening.

In two previously reported intermittent patients of MSUD, maple syrup urine odor was noted during the episode of vomiting and diarrhea or high fever<sup>[13,14]</sup>. In our case, maple syrup odor was never present in his urine. The diagnosis of our case was achieved by a genetic analysis before acute metabolic crisis occurs, underlining the importance of genetic testing in early diagnosis of suspicious cases.

## **CONCLUSION**

Our work adds to the current knowledge of the disease course of intermediate form of MSUD. Our experience on this intermediate MSUD case suggests that a genetic analysis is important for ambiguous cases suspected by metabolic profiling. Our study also alerts clinicians to be cautious of variable clinical phenotypes, metabolic spectrum and medical history to avoid missing of the diagnosis in patients with non-classic mild phenotypes of MSUD.

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