

Case Report

DBT Gene Mutation Among Maple Syrup Urine Disease (MSUD) In Malaysian Population

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Abstract—Maple syrup urine disease (MSUD) is an autosomal recessive genetic disease and an inherited autosomal recessive trait. It is characterised by a deficiency of an enzyme complex, resulting in an excess of branched-chain amino acids (BCAAs), which are toxic to the nervous system. Our four cases generally presented with lethargy and poor feeding weeks after birth. They were all treated for sepsis until the laboratory results showed high levels of BCAAs, which indicated MSUD. Genetic analysis showed that the four cases were homozygous for the *DBT* gene mutation c.1196C>G (p.S399C), a possible founder mutation. All of our cases were managed accordingly, with regular monitoring of the BCAA levels. Dietary support, with infant formulas free of BCAAs, was provided to all four cases with regular follow-up at the paediatric genetic clinic. All cases had spastic diplegic and developmental delays.

Keywords— Maple syrup urine disease; MSUD; BCAA; *DBT* gene; founder mutation

I. INTRODUCTION

Maple syrup urine disease (MSUD) is an inherited autosomal recessive genetic disorder. It was first described in 1954 in a family with four successive affected new-borns who developed progressive neurological disease within the first week of life. MSUD is characterised by a deficiency of a branched-chain alpha-keto acid dehydrogenase (BCKAD) complex, which is required to metabolise the three branched-chain amino acids (BCAAs): leucine, isoleucine, and valine.

The worldwide incidence has been reported to be one in 185,000, and the incidence and prevalence vary among countries. MSUD affects about one in every 180,000 new-borns in the United States [1]. In Karachi, Pakistan, the

incidence is 9.1% among aminoacidopathies [2]. It is more common in populations with a high frequency of consanguinity, and there is no sex predilection.

MSUD is classified into five clinical subtypes: classical, intermediate, intermittent, thiamine responsive, and dihydrolipoyl dehydrogenase deficient. Patients appear normal at birth; however, they may present with feeding difficulties, with progression to lethargy and unresponsiveness. The classical subtype is the most common and severe type, manifesting as dystonia, vomiting, poor feeding, and seizures during the new-born period [3].

MSUD is diagnosed by the presence of clinical features with a high index of suspicion. The biochemical hallmarks of the disorder are an increase in serum BCAAs,

alloisoleucine in plasma, and BCAAs and branched chain ketoacids (BCKAs) in urine. Accumulation of these three types of amino acids and their corresponding alpha-keto acids leads to progressive neurodegeneration and encephalopathy in untreated infants. Without early intervention and treatment, these infants will develop severe and permanent brain damage. They can succumb to death within the first months of life.

Early diagnosis is critical, and dietary intervention should be started immediately to manage MSUD. The two most important aspects of MSUD treatment are long-term management and the treatment of acute metabolic crisis episodes to prevent complications. The goal of treatment is to immediately reduce plasma BCAA and BCKA levels. Dietary restriction of BCAAs is necessary to maintain plasma BCAA concentrations at levels as close to normal as possible. This is essential for restoring and maintaining metabolic homeostasis in patients with MSUD. Here, we present four patients with MSUD who were diagnosed, treated, and monitored in our teaching hospital.

II. CASE REPORTS

A. Case 1

A 13-day-old baby boy was admitted to the neonatal intensive care unit with a history of fever, lethargy, poor feeding, and fits. He is a product of a non-consanguineous marriage, and there is no family history of similar problems. On examination, the baby was in respiratory distress. He had choreoathetoid movements. The baby was treated for sepsis with antibiotics. The results of routine laboratory investigations were normal. A computed tomography (CT) scan of the brain showed cerebral oedema. The plasma amino acid profile showed valine (627 $\mu\text{mol/L}$), isoleucine (459 $\mu\text{mol/L}$), and leucine (3,602 $\mu\text{mol/L}$). Alloisoleucine was detected. Urine organic acid analysis showed marked elevation of 2-ketoisocaproate, 2-keto-3 methyl valerate, and 2-hydroxy isovalerate, with moderate elevation of lactate. The patient was diagnosed with MSUD, and treatment was started immediately. At the time of writing, the patient is undergoing regular BCAA monitoring. Genetic analysis was conducted and showed the homozygous *DBT* gene mutation c.1196C>G (p.S399C). Both parents were tested for the genetic mutation and confirmed as carriers.

B. Case 2

An eight-day-old baby girl was admitted to the neonatal intensive care unit with a history of lethargy, poor feeding, and fits. On examination, the baby was not in respiratory distress. She had opisthotonos and dystonic movements. Her reflexes were brisked. She is the product of a non-consanguineous marriage. There is no family history of similar problems, and the patient was on mixed feeding. She was treated for sepsis with antibiotics. The results of routine laboratory investigations were normal. The plasma amino acid profile showed valine (1,039 $\mu\text{mol/L}$), isoleucine (686 $\mu\text{mol/L}$), and leucine (3,714 $\mu\text{mol/L}$). Alloisoleucine was detected. Urine organic acid analysis showed marked elevation of 2-ketoisocaproate, 2-keto-3 methyl valerate, and 2-hydroxy isovalerate, with moderate elevation of 4-hydroxy phenyl lactate and 2-hydroxy-3 methyl valerate. She was

discharged well with regular BCAA monitoring. Genetic analysis was conducted and showed the homozygous *DBT* gene mutation c.1196C>G (p.S399C). Both parents were tested for the genetic mutation and confirmed as carriers.

C. Case 3

A 30-day-old baby boy presented with poor weight gain, irritability, and upper motor neuron signs. He was treated for meningitis and to rule out inborn metabolic errors. He is a product of a non-consanguineous marriage and the youngest of five siblings. His second brother died at the age of 28 days after being admitted for feeding intolerance and unexplained neurological problems. His other siblings were well. On examination, the baby was in respiratory distress and under nourished. His weight placed him in <3rd percentile, his length placed him in the 3rd–15th percentile, and his head circumference placed him in the 3rd–10th percentile. He was hypertonic, with brisk deep tendon reflexes and absent primitive reflexes. The baby was treated for sepsis with antibiotics. The results of routine laboratory investigations were normal. A CT scan of the brain showed a generalised hypodense appearance of the cerebral white matter, both thalamus, internal capsules, and brainstem, possibly due to ischemic changes. The plasma amino acid profile showed valine (804 $\mu\text{mol/L}$), isoleucine (385 $\mu\text{mol/L}$), and leucine (2,959 $\mu\text{mol/L}$). Alloisoleucine was detected. He was diagnosed with MSUD, and treatment was started immediately. At the time of writing, the patient is undergoing regular BCAA monitoring. Genetic analysis was conducted and showed the homozygous *DBT* gene mutation c.1196C>G (p.S399C). Both parents were tested for the genetic mutation and confirmed as carriers.

D. Case 4

A three-day-old baby boy was admitted to the neonatal intensive care unit with a history of fever, lethargy, and poor feeding. He is a product of a consanguineous marriage; his parents are cousins. There is no family history of similar problems. The patient was on mixed feeding. He was treated for sepsis with antibiotics. The results of routine laboratory investigations were normal. The plasma amino acid profile showed valine (718 $\mu\text{mol/L}$), isoleucine (804 $\mu\text{mol/L}$), and leucine (3127 $\mu\text{mol/L}$). Urine organic acid analysis showed marked elevation of 2-ketoisocaproate, 2-keto-3 methyl valerate, and 2-hydroxy isovalerate, with moderate elevations of acetoacetate, 4-hydroxy phenyl lactate, and 3-hydroxy butyrate. He was diagnosed with MSUD, and treatment was started immediately. At the time of writing, the patient is undergoing regular BCAA monitoring. Genetic analysis was conducted and showed the homozygous *DBT* gene mutation c.1196C>G (p.S399C). Both parents were tested for the genetic mutation and confirmed as carriers.

III. DISCUSSION

MSUD is a potentially life-threatening metabolic disorder. The presentation begins with non-specific symptoms of neurological dysfunction, seizures, irritability, and poor feeding. These are followed by focal neurological signs, such as abnormal movements, increasing spasticity, and coma. If left untreated, patients can succumb to brain damage and death within weeks.

MSUD is inherited in an autosomal recessive manner. It results from mutations in one of three different genes: *BCKDHA*, *BCKDHB*, and *DBT* [4]. These mutations result in the absence or decreased activity of BCKAD enzymes, which are responsible for breaking down BCAAs. The accumulated BCAAs produce toxic by-products (ketoacids), resulting in metabolic acidosis (Figure 1).

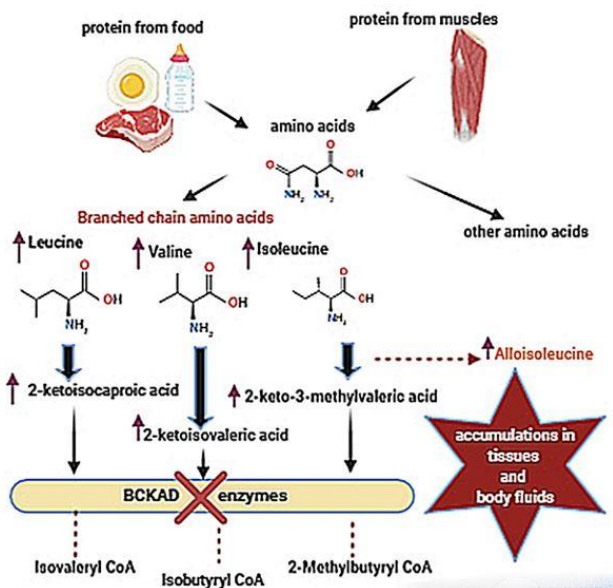


Figure 1. Metabolic pathway of BCAA (valine, leucine, and isoleucine) degradation. The degradation of the essential BCAAs is impaired by BCKAD deficiency.

All four cases presented to our hospital in infancy (Table 1). Based on the onset and presentation of these cases, they were categorised as classical MSUD. At birth, patients with classical MSUD seem normal; they develop symptoms weeks after birth. Generally, the clinical manifestation of MSUD depends on the severity of the BCKAD deficiency. Classical MSUD usually involves less than 2% enzyme activity [5]. Patients present with lethargy, inactivity, poor feeding, and irritability. Their urine smells like maple syrup or burnt sugar due to ketonuria in the first weeks of life.

In Malaysia, clinical suspicion and the detection of inborn errors of metabolism play an important role in diagnosis. The initial step for the detection of BCAAs or any amino-acidopathies is urine sample analysis. In the past, urine samples were analysed using thin-layer chromatography, which was tedious and laborious. What's more, the results were often inconclusive because of the presence of other substances in the urine (e.g., drugs), and alloisoleucine (the pathognomonic marker for MSUD) cannot be detected using this method [5].

New methods have been developed for screening and confirmation. The introduction of new-born screening using tandem mass spectrometry (TMS) in the early 1990s improved the sensitivity and specificity of the detection of inborn metabolic errors [6]. TMS is used to analyse dry blood spotted onto filter paper, and it is one of the most effective MSUD detection methods. Several inherited disorders can now be identified via new-born screening, and with this screening the numbers have increased more than five-fold [7, 8].

In all our cases, a high index of suspicion of a genetic disease led to the paediatricians submitting blood samples for testing to rule out inborn errors of metabolism. Blood was spotted on filter paper and sent to a centre that specialises in screening for inborn errors of metabolism using TMS. The results of all four cases suggested MSUD. Further quantification via high-performance liquid chromatography showed elevated levels of BCAAs (Figure 3) compared to the normal range (Figure 2) and the presence of alloisoleucine (Figure 4). Work-up for mutational analysis was performed for our four cases and both their parents.

Mutational analysis for MSUD among the Malaysian population has shown the *BCKDHB* gene to be the most affected gene compared to the *BCKDHA* and *DBT* genes [9]. In all of our cases, the patients had a similar mutation in the *DBT* gene. They were all found to have a homozygous c.1196C>G (p.S399C) genotype. In all cases, both parents were carriers of the same mutation.

Physiologically, the *DBT* gene encodes the dihydrolipoyl transacylase (E2) subunit, an important specific protein in the enzyme complex. This enzyme complex is organised as a cubic core consisting of 24 identical E2 subunits with E1 and E3 (a specific kinase and a specific phosphatase, respectively) attached through ionic interaction. These enzymes are responsible for the regulation of the BCKD complex. Mutations found in these genes are thought to cause disease via the creation of a premature termination codon. *Silico* analysis has suggested that this mutation results in damage to protein function [9]. Mutations in the *DBT* gene affect the production of E2 and regulation of the BCKD enzyme complex, resulting in the inability to metabolise the essential BCAAs leucine, isoleucine, and valine [15].

The mutation detected in our cases is a new gene mutation that has been observed among ethnic Malays. This finding suggests the possibility of a founder mutation in the *DBT* gene; however, further tests need to be conducted to confirm the founder effect among this population [9]. Founder mutations in the *DBT* gene have been reported among the Filipino population [10] and in the general population of the Paiwan Austronesian Aboriginal tribe in southern Taiwan [11]. In addition to the founder mutations in the *DBT* gene, founder mutations have also been identified in the *BCKDHA* and *BCKDHB* genes in three different populations [12-14].

A founder mutation is a mutation that appears in the DNA of one or more individuals who are founders of a distinct population. This mutation is initiated by changes in the DNA that can be passed down to subsequent generations. The mutations originate in long stretches of DNA on a single chromosome; indeed, the original haplotype is the whole chromosome; indeed, the original haplotype is the whole chromosome. As generations progress, the proportion of the haplotype that is common to all carriers of the mutation is shortened (due to genetic recombination). This shortening allows scientists to roughly estimate the age of the mutation [12].

TABLE 1.
Summary of the four cases diagnosed with MSUD: Plasma BCAA values and organic acid analysis findings at presentation.

Patient	Sex	Age at presentation	Clinical presentation	Plasma amino acid (µmol/L)	Urine organic acid	Subsequent clinical progress	Outcome	Clinical phenotype	Mutation site	Exon/intron	Genetic subtype	Genetic analysis
1	M	13 days	Fever, lethargy, poor feeding, and fits	Val: 627 Leu: 3,602 Ile: 459	2-ketoisocaproate, 2-keto-3 methyl valerate, 2-hydroxy isovalerate, and lactate	Gross motor delay Spastic diplegic Bilateral ankle contracture	Survived On specialised milk and thiamine supplement	Classical	HM	Ex-9	E2	<i>DBT</i> gene homozygous c.1196C>G, p.S399C
2	F	8 days	Lethargy, poor feeding, and fits	Val: 1,039 Leu: 3,714 Ile: 686	2-ketoisocaproate, 2-keto-3 methyl valerate, 2-hydroxy isovalerate, 4-hydroxy phenyl lactate, and 2-hydroxy-3 methyl valerate	Epilepsy Mild developmental delay Spastic diplegic	Survived On specialised milk and thiamine supplement	Classical	HM	Ex-9	E2	<i>DBT</i> gene homozygous c.1196C>G, p.S399C
3	M	30 days	Poor weight gain and upper motor neuron signs	Val: 804 Leu: 2,959 Ile: 385	Not available	Global developmental delay Spastic diplegic	Survived On specialised milk and thiamine supplement	Classical	HM	Ex-9	E2	<i>DBT</i> gene homozygous c.1196C>G, p.S399C
4	M	3 days	Fever, lethargy, and poor feeding	Val: 718 Leu: 3,127 Ile: 804	2-ketoisocaproate, 2-keto-3 methyl valerate, 2-hydroxy isovalerate, 4-hydroxy phenyl lactate, and 3-hydroxy butyrate	Epilepsy Global developmental delay Spastic diplegic Distal renal tubular acidosis (type 1)	Survived On specialised milk and thiamine supplement	Classical	HM	Ex-9	E2	<i>DBT</i> gene homozygous c.1196C>G, p.S399C

Val = Valine; Leu = Leucine; Ile = Isoleucine; F = Female; M = Male; HM = homozygous

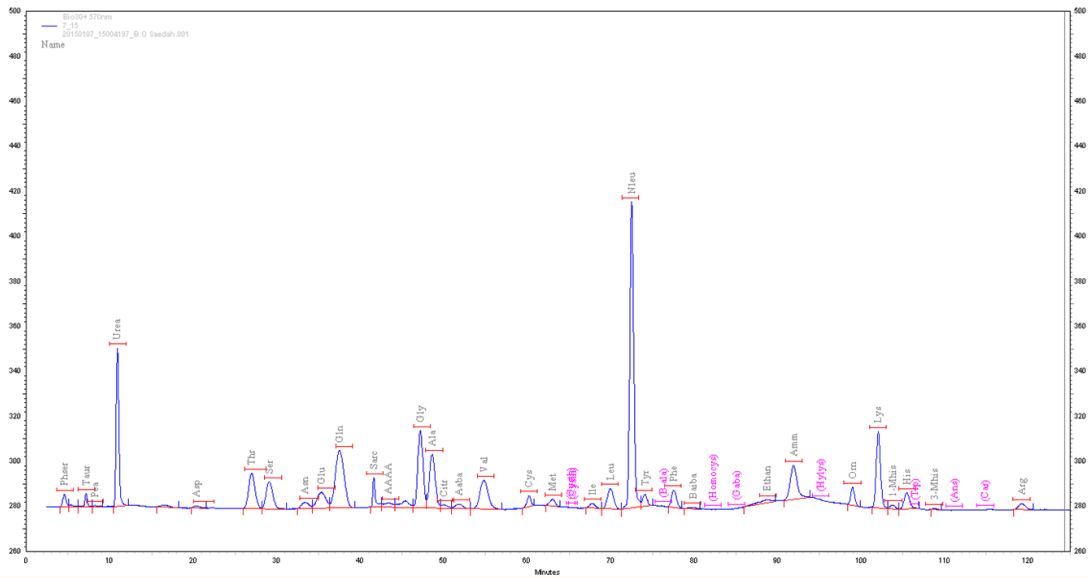


Figure 2. Plasma amino acid levels of a normal person.

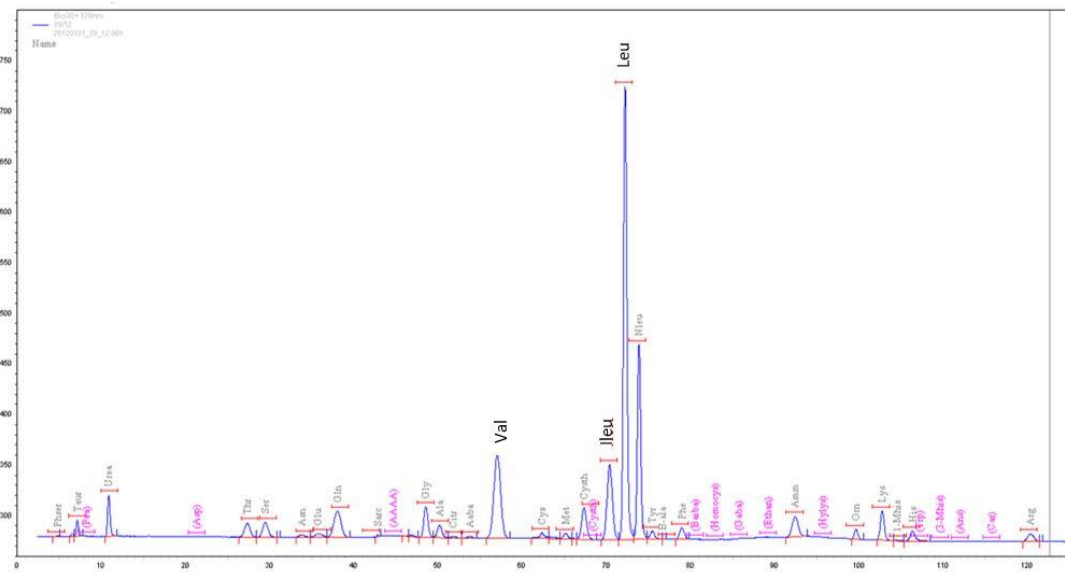


Figure 3. Plasma amino acid levels of one of our patients with MSUD showed high leucine, isoleucine, and valine levels.

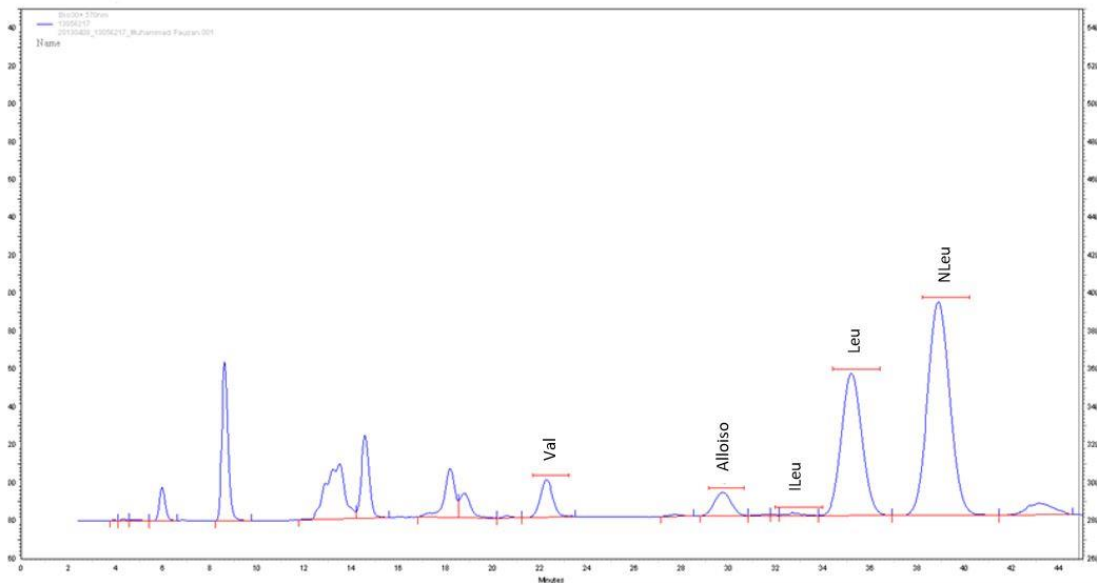


Figure 4. Chromatogram showing alloisoleucine detection in one of our patients. Alloisoleucine is a by-product of isoleucine transamination.

There is an apparent correlation between *DBT* gene mutations and thiamine-responsive phenotype. The biochemical mechanism of the thiamine response in this type of mutation is currently under investigation [15]. A study in South India, however, reported that patients with *DBT* gene mutations were non-responsive to thiamine supplementation and had worse prognoses than patients who did not have such mutations [16].

Based on the diagnosis and acute condition presented, all our cases were attended to and managed immediately. In the management of the acute state, the aim was to remove the BCAAs and their BCKAs. Peritoneal dialysis and hemofiltration are two of the most effective methods for critically ill infants; however, none of our cases underwent either of these procedures. Instead, they were all treated for sepsis with antibiotics, followed by dietary restriction of BCAAs to achieve metabolic homeostasis. BCAA levels were repeatedly measured and monitored during the crisis, with strict monitoring of other biochemical parameters.

Our cases were discharged well, and they were started on specialised milk and thiamine supplements. Support and counselling sessions were provided to the parents. However, despite the restrictions on the consumption of certain proteins and the specialised diet, the risk of metabolic crisis still exists for our cases due to the inadequate supply of specialised milk and co-existing infections. They have been managed accordingly during each crisis, with regular follow-ups at our genetic clinics. Since diagnosis of MSUD is time sensitive, a delay in diagnosis is invariably associated with learning disability. Correlation between phenotype and genotype does not depend on the clinical situation; however, neurological deterioration is directly associated with early diagnosis and treatment [17]. All of our cases had some form of mild-to-moderate learning difficulties, and this disability was further enhanced with episodes of crisis that had caused global developmental delay.

IV. CONCLUSION

Several types of mutations associated with MSUD have been reported in Malaysia. All of our patients were in the category of classical MSUD with confirmed *DBT* gene mutation and the possibility of founder mutation. They presented early during infancy and were treated for sepsis. Despite the administration of aggressive treatment to prevent complications, all cases had developmental delay. Protein restrictions via a specialised diet and artificial formulas were provided to all our cases to ensure they received the necessary nutrients for growth and development. Their BCAA levels are being evaluated regularly, and their growth and development are being closely monitored.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

CONSENT TO PARTICIPATE

Written informed consent for the present case reports were obtained from the patient's parents.

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