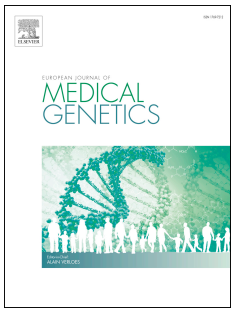

# Eleven novel mutations and clinical characteristics in seven Chinese patients with thiamine metabolism dysfunction syndrome

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**Conflict of interest**

All authors declare none conflict of interest.

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# Eleven novel mutations and clinical characteristics in seven Chinese patients with thiamine metabolism dysfunction syndrome

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**Running title:** Eleven novel mutations on thiamine metabolism dysfunction syndrome

**Abstract**

Thiamine metabolism dysfunction syndrome (THMD) comprises a group of clinically and genetically heterogeneous encephalopathies with autosomal recessive inheritance. Four genes, *SLC19A3*, *SLC25A19*, *SLC19A2*, and *TPK1*, are associated with this disorder. This study aimed to explore the clinical, biochemical and molecular characteristics of seven Chinese patients with THMD. Targeted next-generation sequencing of mitochondrial DNA and nuclear DNA was used to identify the causative mutations. The patients presented with subacute encephalopathy between the ages of 1 to 27 months. Brain magnetic resonance imaging (MRI) revealed abnormalities in the basal ganglia, indicating Leigh syndrome. Urine α-ketoglutarate in five patients was elevated. In four patients, five novel mutations (c.1276_1278delTAC, c.265A>C, c.197T>C, c.850T>C, whole gene deletion) were found in *SLC19A3*, which is associated with THMD2. In two patients, four novel mutations (c.194C>T, c.454C>A, c.481G>A, and c.550G>C) were identified in *SLC25A19*, supporting a diagnosis of THMD4. In one patient, two novel mutations (c.395T>C and c.614-1G>A) were detected in *TPK1*, which is indicative of THMD5. The patients received thiamine, biotin, and symptomatic therapy, upon which six patients demonstrated clinical improvement. Our findings expanded the phenotypic and genotypic spectrum of THMD, with eleven novel mutations identified in seven Chinese patients. Early diagnosis and treatment have a significant impact on prognosis.

**Key words:** Thiamine metabolism dysfunction syndrome (THMD), Leigh syndrome, SLC19A3, SLC25A19, TPK1, Clinical exome sequencing

## 1. Introduction

Thiamine metabolism dysfunction syndrome (THMD), which results from a deficiency of thiamine metabolism, comprises a group of rare, clinically and genetically heterogeneous encephalopathies with autosomal recessive inheritance (Banka et al., 2014). Four genes, *SLC19A3*, *SLC25A19*, *SLC19A2*, and *TPK1*, are linked to this disorder. Based on the defective genes and phenotypic traits, THMD is classified into five types: THMD1 [Online Mendelian Inheritance in Man (OMIM) #249270], caused by mutations in *SLC19A2*; THMD2 (OMIM #607483), caused by mutations in *SLC19A3*; THMD3 (OMIM #607196, microcephaly, Amish-type) and THMD4 (OMIM #613710, progressive polyneuropathy type), caused by the mutations in *SLC25A19*; and THMD5 (OMIM #614458, episodic encephalopathy type), caused by mutations in *TPK1* (Brown, 2014). The clinical course of patients with THMD is usually progressive, leading to severe disability and even death if left untreated.

To date, 38 mutations in *SLC19A3*, 8 in *SLC25A19*, and 13 in *TPK1* have been reported (Human Gene Mutation Database, HGMD) globally, however only two reports on *SLC19A3* (Klein et al., 2004; Wen et al., 2019) and two on *TPK1* (Fraser et al., 2014; Zhu et al., 2019) have been published on the Chinese population. In contrast, there have been just four studies on *SLC25A19* mutations associated with THMD4 (Spiegel et al., 2009; Ortigoza-Escobar et al., 2017; Bottega et al., 2019; Gowda et al., 2019) worldwide, with none documenting the Chinese population. Leigh syndrome and Leigh-like syndrome are mitochondrial disorders with considerable clinical and genetic heterogeneity caused by either the mitochondrial or nuclear genome. Here, we present seven Chinese cases of Leigh syndrome due to THMD,

expanding the clinical and molecular spectrum of the disease.

## 2. Materials and Methods

### *2.1 Patients*

Seven unrelated patients (one boy and six girls) from different Chinese families were enrolled in this study. They were admitted to our hospital aged from 1 month to 4 years and 3 months between June 2008 and June 2019 with the suspected diagnosis of Leigh syndrome. They presented with subacute encephalopathy from the ages of 1 month to 27 months with clinical manifestations including seizures, hypotonia, dyskinesia, and developmental regression. All of the parents were non-consanguineous and without positive family history. The clinical data of the patients were collected.

This study was approved by ethics committee at Peking University First Hospital. The informed consents were obtained from all the patients' legal guardians.

### *2.2 Routine laboratory, metabolic, and auxiliary examinations*

Routine blood and urinary tests were conducted. Serum lactate, pyruvic acid, ammonia, glucose, creatine kinase, vitamin D, liver and renal function were evaluated. Colorimetric analysis was used to determine blood biotin and biotinidase activities. Tandem mass spectrometry was applied to determine serum amino acids and acylcarnitine. The results were analyzed using ChemoView software. Gas chromatography-mass spectrometry and the Inborn Errors of Metabolism Screening System were used to analyze urine organic acids. Cranial MRI was performed on each patient.

### *2.3 DNA preparation and targeted next-generation sequencing*

Genomic DNA was extracted from the peripheral blood leukocytes of all patients and their parents. Genes targeted to the mitochondrial DNA (mtDNA; the 16569-base pair mitochondrial genome) were firstly sequenced. Based on a literature search of online databases, we used a custom-designed gene panel containing 246 nuclear genes of interest associated with mitochondrial diseases, which included *SLC19A3* and *TPK1*, but not *SLC25A19* or genes related to biotin deficiency-related disorders. A clinical exome panel, including 4813 genes associated with known phenotypes, was also designed with reference to the literature (Jamal et al., 2013).

Next-generation sequencing (NGS) was performed as described in our previous study (Xu et al., 2017). We implemented the following strategy: mtDNA was initially captured , then the mitochondrial disease-related nuclear gene panel, clinical or whole exome sequencing was eventually performed if nothing was found .

### *2.4 Confirming of the related mutations*

The mutations detected from the seven patients were confirmed by Sanger sequencing. Then, parental origin was performed. The large fragment deletion was confirmed by SNP-array. The results were compared with the *SLC19A3* (NM_025243.3), *SLC25A19* (NM_021734.4), and *TPK1* (NM_022445.3) sequences in GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Regarding the novel mutations, the corresponding amplicons were amplified from 200 normal control samples.

### *2.5 Prediction of the effect and conservation analysis of the novel mutations*

Multiple sequence alignment studies were performed to verify the degree of conservation using the UCSC Genome Bioinformatics Database. Mutation taster, PolyPhen-2, and SIFT programs were used to predict the impact of missense mutations on protein function.

### *2.6 Follow-up study*

All seven patients were enrolled into a simple follow-up study by telephone survey or an outpatient visit. Information on seizures, convulsions, inductive factors, times of attacks, motor and cognitive development, and other neurological symptoms were collected. General laboratory tests, metabolic studies and brain MRI were performed.

## 3. Results

### *3.1 Clinical features, laboratory examinations, brain MRI findings, and follow-up observations (Table 1)*

Seven patients were born at term after normal pregnancy and delivery. They presented from the age of 1 month to 2 years and 5 months with recurrent episodes of encephalopathy, frequently triggered by febrile disease. Disease onset in 4 of the patients was during the infant period, while the disease developed in the other three after one year of age. Trigger factors before attacks such as fever, trauma and vaccination were observed. All patients reached normal developmental milestones until their first attack. They presented with varied movement disorders after onset. Abnormal signals mainly in basal ganglia were observed in all patients, indicating Leigh syndrome. Blood lactate ranged from 1.1 mmol/L to 12.7

mmol/L. Levels of urinary organic acids of five patients were examined, and their urine α-ketoglutarate was found to be elevated. Thiamine, biotin, L-carnitine, coenzyme $Q_{10}$ supplements and symptomatic treatment were administrated after diagnosis. Thiamine was given at a dose of 100 mg/d before genetic diagnosis. Once we got the genetic result, thiamine was increased to at least 10 mg/kg/d. One case (P2) got nearly complete recovery, one died and five cases (P1, P3, P4, P5, and P6) improved. We had submitted all the new or exceptional DNA variants or CNVs mentioned in this article to ClinVar, and the accession numbers could be found in table 2.

### *3.2 Patient 1*

P1 is female and had normal development until the age of 4 months. From the age of 4 months, she developed subacute encephalopathy which manifested as focal seizures, infantile spasms, lethargy, feeding difficulty and neurodevelopmental regression. The seizures occurred daily. Thiamine or biotin was not given in local hospital. When she visited us at 1-year old, severe psychomotor retardation and microcephaly (42 cm, < -2SD) were observed. She could not raise her head or kick or recognize her family members. She presented dystonia, especially when crying. Serum lactate was 2.2 mmol/L. Hypsarrhythmia was observed on electroencephalography. Brain MRI revealed abnormal signals in the caudate nucleus, putamen, globus pallidus, thalamus, midbrain, and cerebellar hemisphere with a thin corpus callosum and progressive cerebral atrophy, indicating the diagnosis of Leigh syndrome (Figure 1). Thiamine and biotin were given at dose of 100 mg/d and 5 mg/kg/d, respectively. After treatment, she improved progressively with a significant reduction in seizure frequency.

Currently, she is 2 years and 6 months old. She is still unable to raise her head or sit. In addition, she exhibits horizontal nystagmus, decayed teeth, dysphagia, moderate hypotonia, and microcephaly.

### *3.3 Patient 2*

P2 is female and developed general muscle weakness on the second day of fever when she was 2 years and 5 months old, approximately half a month after measles vaccination. Then, inability to walk, week cry, dysarthria, dystonia, ptosis, and positive bilateral Babinski signs were observed. She was admitted as suspected encephalitis to a local hospital and treated as such. She recovered gradually and had fully recovered within 2 weeks. The second attack occurred at the age of 4 years and 2 months. Most of the manifestations resembled those at the first attack, but no fever occurred as a trigger. She exhibited near complete recovery a week later. When she visited us at the age of 4 years and 3 months, she presented with a recurrent feeling of numbness in her hands and feet. Cranial MRI showed abnormal signals in the bilateral caudate nucleus, putamen, and cortex. Leigh syndrome was suspected, and thiamine (100 mg/d) and biotin (5 mg/kg/d) was given.

Significant improvement was observed after treatment. The feeling of numbness gradually disappeared. Currently, she is a junior school student with excellent academic results.

### *3.4 Patient 3*

P3 is female with progressive recurrent encephalopathy. She presented dyskinesia and developmental regression triggered for the first time by fever at the age of 8.5 months. Her

brain MRI showed symmetrical T2WI and FLAIR hyperintensity in the globus pallidus and putamen. No special treatment was given in local hospital. She exhibited developmental delay, paroxysmal opisthotonos, encephalopathy, hypotonia, and a brisk tendon reflex when she visited us at 9 months. Thiamine was given at a dose of 100 mg/d. She recovered incompletely within a month, exhibiting slightly delayed motor development in left leg. Her motor skills recovered gradually, and she could walk a short distance without support at 15 months. At 4 years of age, dyskinesia occurred again without any trigger. She could not sit, and presented hypotonia. The motor symptoms improved within 3 weeks. She underwent another two encephalopathy episodes brought on by fever at 7 and 9 years of age, respectively. During these two attacks, the course was similar to that of the second episode. Dystonia was also observed. Meanwhile, the genetic result showed *SLC19A3* deficiency, so biotin was administered at a dose of 10 mg/kg/d. Her urine glutaric acid (37.6 mmol/mol Cr vs. the normal range of 1.9–4 mmol/mol Cr) and α-ketoglutarate (139 mmol/mol Cr vs. the normal range of 26.1–102.9 mmol/mol Cr) levels were slightly elevated. Brain MRI showed abnormal signals in the globus pallidus, putamen, caudate nucleus, and cerebellum.

Currently, she is 11 years old, bedridden and unable to walk without support, with hypertonia, torsion spasm, equinus. She can speak a few word, but not a sentence.

### *3.5 Patient 4*

P4 is female with normal developmental milestones until she suffered severe acute encephalopathy at the age of 1 month. She presented drug-resistant partial seizures with or without fever, poor intake, and hypotonia. She was admitted to the neonatal intensive care

194 unit and started on phenobarbital and antibiotics. Then, her condition deteriorated quickly on
195 the second day as she required mechanical ventilation. Thiamine and biotin was given
196 immediately after diagnosis was confirmed by MRI.
197 Her serum lactate was 3.8-12.7 mmol/L (normal range, 0–2 mmol/L). Mildly elevated urine
198 α-ketoglutarate (229 mmol/mol Cr vs. the normal range of 26.1–102.9 mmol/mol Cr) were
199 found. Video-electroencephalography (V-EEG) showed mild abnormal neonatal EEG with
200 multifocal spikes and low-frequency waves. Brain MRI revealed abnormal signals in the
201 globus pallidus, putamen, and caudate nucleus. Leigh syndrome was diagnosed. She got
202 intractable epilepsy even given thiamine and biotin treatment.
203 She died from cardiopulmonary respiratory failure at 2 months and a half.
204
205 ***3.6 Patient 5***
206 P5 is male and suffered recurrent encephalopathy after the age of 8 months, when he
207 presented febrile seizure concomitant with developmental regression. He could not raise his
208 head, roll over or sit. Two similar attacks occurred at the age of 13 months and 14 months.
209 When he visited us at his age of 18 months, he had severe developmental delay.
210 His serum lactate was elevated to 3.51 mmol/L (normal range, 0–2 mmol/L). His blood biotin
211 level was significantly reduced (70 pg/mL vs. the normal range of >200 pg/mL). The blood
212 biotinidase activity was normal. Markedly elevated urine α-ketoglutarate (789.1 mmol/mol Cr
213 vs. the normal range of 26.1–102.9 mmol/mol Cr) were found. Brain MRI revealed abnormal
214 signals in the putamen and caudate nucleus. The nerve conduction velocity was not done.
215 Leigh syndrome and biotin deficiency were diagnosed. He could walk independently at the

age of 2 years and 10 months after treatment.

Currently, he is 3 years old with normal intelligence and slight motor developmental delay.

### *3.7 Patient 6*

P6 is female and presented recurrent encephalopathy since the age of 14 months, when she suffered tremor and developmental regression. She showed gradual improvement 2 weeks later. When she was admitted at the age of 19 months, she could not walk and presented with developmental delay. Urinary α-ketoglutarate was elevated (968.4 mmol/mol Cr vs. the normal range of 26.1–102.9 mmol/mol Cr). Brain MRI revealed symmetrical abnormal signals in the caudate nucleus and putamen. Six months later after treatment, her brain MRI showed nearly complete recovery (Figure 2). No further attacks occurred.

Currently, she is 2 years and 3 months old with normal intelligence and slight motor developmental delay.

### *3.8 Patient 7*

P7 is female and presented with recurrent encephalopathy since the age of 8 months at which time she suffered slow progressive motor developmental delay. At the age of 16 months, she developed tremor in her legs. She could walk at the age of 18 months. However, 1 month later, she became unable to walk after a tumble until the age of 2 years. When she visited us at the age of 2 years and 1 month, she presented with moderate intellectual and motor disabilities. Urinary α-ketoglutarate was elevated to 390.3 mmol/mol Cr (normal range, 26.1–102.9 mmol/mol Cr). Cranial MRI showed bilateral abnormal signals mainly in the caudate

nucleus, putamen, thalamus, and cerebellar hemispheres, with mild progressive atrophy of the cerebellum (Figure 3). Rapid improvement was observed 5 days after 10 mg/kg/d thiamine supplementation. However, after a trauma at the age of 4 years and 5 months, she lost the ability to walk again. At the age of 5 years, she was hospitalized for dysarthria, lethargy, and paroxysmal muscular weakness after a febrile illness. Her symptoms significantly improved 10 days after the thiamine supplement (15 mg/kg/d).

Currently, she is 5 years and 3 months old. She can walk a short distance unaided with a spastic gait. She can speak simple words related to the daily life.

### *3.9 Molecular analysis*

Twelve mutations were identified from seven patients, eleven of which were novel. There were six mutations of the *SLC19A3* in total were detected in patients from 1 to 4 (Table 2), of which five were novel comprising four heterozygous mutations and one homozygous mutation, supporting the diagnosis of THMD2. The whole exome sequencing results showed that P4 had a suspected 130Kb homozygous fragment deletion (chr2q36.3:228482780-228609111) inclusive of the whole *SLC19A3*, which was then verified by SNP-array. Four novel heterozygous mutations in *SLC25A19* were detected in P5 and P6 supporting the diagnosis of THMD4. Two heterozygous mutations in *TPK1* were detected in P7, supporting the diagnosis of THMD5. All eleven mutations were inherited from their unaffected parents. None of the novel mutations were found in 200 normal controls. And the 130Kb fragment deletion was neither reported nor found in Decipher, CAGdb or Pubmed database.

### *3.10 Prediction of effects of the eleven novel mutations*

Eight novel mutations (Table 2) (c.1276_1278delTAC, c.265A>C, c.197T>C, and c.850T>C in *SLC19A3*; c.454C>A, c.481G>A, and c.550G>C in *SLC25A19*; and c.395T>C in *TPK1*) were predicted to be deleterious by at least two programs (Mutation taster, SIFT, and Polyphen 2) (Tables 3-4). The c.194C>T variation was predicted to be deleterious by Mutation taster alone and to be a polymorphism by the other two programs, but it is located in a highly conserved region.

## 4. Discussion

THMD, which usually manifests as acute or subacute encephalopathy, is caused by a deficiency of thiamine metabolism, with elevated excretion of lactic acid and a-ketoglutaric acid as a supportive criteria (Toyoshima et al., 2005; Ortigoza-Escobar et al., 2017). Patients with this disorder show a variable response to the administration of thiamine and/or biotin. Thiamine is recommended at a dose of 10-40 mg/kg/day for *SLC19A3* deficiency and 30 mg/kg/day for *TPK1* deficiency, whereas biotin is recommended at dose of 5-10 mg/kg/day for *SLC19A3* deficiency(Ortigoza-Escobar et al., 2016). Four *SLC25A19* related THMD4, thiamine at a dose of 400-600 mg/d were effective in five patients(Ortigoza-Escobar et al., 2017). Neurological involvement occurs predominantly because of mutations in three genes, *SLC19A3*, *SCL25A19*, and *TPK1*, resulting in the disorders of THMD2, THMD3 and THMD4, and THMD5, respectively. In many patients, the onset or recurrence of the condition is triggered by febrile disease, vaccines, and trauma (Kevelam et al., 2013).

Our patients manifested subacute encephalopathy of infantile onset with heterogeneous clinical features and similar brain MRI findings. Clinical features mainly included dystonia, hypotonia, ataxia, seizures, and developmental delay, most with trigger events and elevated a-ketoglutaric acid in urine. Brain MRI lesion mainly involved in caudate, putamen, globus pallidus, some in thalamus, brain stem, and cerebellum. Both of clinical features and brain MRI findings met the clinical criteria of THMD (Ortigoza-Escobar et al., 2017). Leigh syndrome was diagnosed and mitochondrial disorders were suspected at first. Patients from 2 to 7 experienced triggers before attacks. According to the results of genetic studies, they were diagnosed with different types of THMD.

THMD2, also termed biotin-thiamine-responsive basal ganglia disease, manifests as encephalopathy with dysphagia, dysarthria, external ophthalmoplegia, even coma and death (Debs et al., 2010). Biotin-thiamine-responsive basal ganglia disease was first described by Ozand *et al*. in 1998 (Ozand et al., 1998). *SLC19A3* was mapped to 2q36.3. *SLC19A3* mutations were found to be the cause of the biotin-thiamine-responsive basal ganglia disease by Zeng *et al*. in 2005 (Zeng et al., 2005). Meanwhile, mutations in the *SLC19A3* also underlie phenotypes such as neonatal lactic acidosis, Wernicke's-like encephalopathy, early childhood fatal Leigh syndrome, and early infantile lethal encephalopathy (Toyoshima et al., 2005; Yamada et al., 2010; Gerards et al., 2013; Kevelam et al., 2013).

Patients from 1 to 4 had *SLC19A3* defects with varied clinical features and nonspecific biochemical findings, except slightly elevated urine α-ketoglutarate levels in P3 and P4. P1 developed infantile epileptic encephalopathy similar to that described by Yamada *et al*. (Yamada et al., 2010). She displayed remarkable dystonia. Thiamine and biotin administration

had a partial effect even though the treatment was delayed. P2 exhibited late-onset acute episodic encephalopathy and dystonia. She underwent complete spontaneous recovery in 1-2 weeks, and the lesions on brain MRI showed a remarkable improvement after thiamine and biotin administration. The course of the disease was mild. This finding suggested that a later onset may indicate a better prognosis and a better response to treatment. The trigger factors for P3 were febrile illness and vaccination. She presented a chronic, progressive disease course with each attack despite thiamine and biotin therapy from the age of 2 years and 1 month. Whether she was responsive to thiamine and biotin remains uncertain because thiamine administration was delayed. P4 also had a febrile trigger factor, she manifested an acute course in accordance with the phenotype of early infantile lethal encephalopathy as reported (Alfadhel, 2017).

For *SLC25A19* associated disorders, there are two phenotypic entities. One is THMD3, also called microcephaly, Amish-type, which is characterized by severe lethal congenital microcephaly with severe elevated lactic acid and α-ketoglutaric acid levels, and the patient died in the first year of life (Siu et al., 2010). The other, also known as progressive polyneuropathy type, is THMD4 which is characterized by severe congenital microcephaly, death within the first year, and severe 2-ketoglutaric aciduria. Only eight consanguineous patients with neuropathy and bilateral striatal necrosis from four consanguineous families have been reported (Spiegel et al., 2009; Ortigoza-Escobar et al., 2017; Bottega et al., 2019; Gowda et al., 2019). In this study, P5 and P6 presented with acute encephalopathy and bilateral striatal lesions triggered by febrile illness with normal early developmental milestones. Their clinical, biochemical, and brain MRI findings were similar to those cases

reported (Spiegel et al., 2009; Bottega et al., 2019; Gowda et al., 2019). However, in our study, these two patients came from different, non-consanguineous families. P5 was found to have biotin deficiency, which may underlie responsiveness to thiamine and biotin. Hence, these two cases expand the phenotypic spectrum of THMD4.

Notably, thiamine was given at a dose of 100 mg/d initially in some patients, less than the recommended dose 10 mg/kg/d. Once we got the genetic results, the dose was added to 10 mg/kg/d at least .

The patients with mutations in *TPK1* exhibited complex clinical manifestations, such as feeding difficulties, encephalopathy, psychomotor regression leading to a severe developmental delay, and progressive dystonia (Fraser et al., 2014). Consistently, P7 presented with motor developmental delay, recurrent encephalopathy, normal plasma lactic acid, elevated urine α-ketoglutarate, and abnormal brain MRI signals mainly in the basal ganglia. THMD was confirmed by genetic analysis. With thiamine supplementation, she showed a significant improvement.

*SLC19A3* encodes a ubiquitous 496-amino acid thiamine transporter that plays an essential role in cerebral thiamine transport (Zeng et al., 2005). *SLC25A19*, located at chromosome 17q25.1, encodes a 320-aminio acid mitochondrial thiamine pyrophosphate transporter that mediates thiamine pyrophosphate uptake into mitochondria (Kang and Samuels, 2008). *TPK1*, located at chromosome 7q35, encodes the 243-amino acid thiamine pyrophosphokinase 1 protein (TPP), which catalyzes the conversion of thiamine to thiamine pyrophosphate. It is mainly expressed in the small intestine and kidney, where it mediates thiamine absorption and reabsorption (Zhao et al., 2001). THMD occurs because of the deficiencies in these genes.

In this study, we identified 11 novel mutations associated with three types of THMD using NGS. Of the 11 novel mutations, one was a small deletion, one was large deletion, eight were missense mutations, and one was splicing mutation. The small deletion and eight missense mutations occurred in highly conserved regions (Tables 3-5). They were predicted to be damaging by at least two of the programs of Mutation taster, SIFT, and Polyphen 2, except for A65V in *SLC25A19*. However, A65V is not a single-nucleotide polymorphism, and it is located in a highly conserved region, also with a rather low frequency in the 1000 Genomes Brower Database, which indicates its pathogenicity. The c.614-1G>A mutation in *TPK1* occurred at a splicing site and leads to the abnormal formation of TPP, confirming its pathogenicity. The whole *SLC19A3* deletion may lead to the loss of gene function. All the novel mutations had serious influences on the function of their protein products, probably leading to the disease.

Targeted NGS using gene panels has been increasingly applied for genetic diagnoses over the past few years. Compared to whole exome sequencing, clinical exome sequencing is cheaper and its results are easier to interpret. It is also faster and more efficient in patients with uncertain diagnostic considerations, even though restricted target sequencing has higher accuracy and deeper coverage (Okazaki et al., 2016). Herein, we applied targeted gene capture sequencing of a mtDNA panel and a mitochondrial diseases-related nuclear gene panel to detect the suspected genes. However, P5 and P6 were missed because *SLC25A19* was not included in the panel, and they were identified by the following clinical or whole exome panel. Clinical and whole exome sequencing is an efficient diagnostic tool in patients with Mendelian disorders, especially in those with complicated heterogeneous diseases like

comparatively complicated suspected mitochondrial diseases.

## Conflict of interest

All authors declare none conflict of interest.

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**Figure legends**

**Figure 1** Cranial MRI of P1 with *SLC19A3* deficiency at the age of 5 months.

(a, b, c): Axial section of T1-weighted images (T1WI) of the brain. The images showed bilateral symmetrical hypointensity signals in cerebellar hemisphere, midbrain, basal ganglia area (caudate nucleus, putamen, and globus pallidus), and thalamus, with thin corpus callosum.

(d, e, f): Axial section of T2-weighted images (T2WI) of the brain. The images showed bilateral symmetrical hyperintensity signals in cerebellar hemisphere, midbrain, basal ganglia area (caudate nucleus, putamen, and globus pallidus), and thalamus, with thin corpus callosum.

(g, h, i): Axial section of fluid-attenuated inversion recovery (FLAIR) of the brain. The images showed bilateral symmetrical hyperintensity signals in midbrain, while hypointensity signals in cerebellar hemisphere, basal ganglia area (globus pallidus and putamen), and thalamus, with thin corpus callosum. Low signals in globus pallidus and putamen were surrounded by high signals.

**Figure 2** Cranial MRI of P6 with *SLC25A19* deficiency.

(a, b): FLAIR and T2WI of the brain on the seventh day of the attack, when she was 15 months old. The images showed bilateral symmetrical hyperintensity signals in the head of the caudate nucleus and putamen.

(c): Axial section of T2WI of the brain at the age of 18 months, 2 months after the attack and without thiamine supplementation. The abnormal signals had reduced somewhat.

(d, e): Axial section of FLAIR and T2WI of the brain on the seventh day of the attack, when

she was 2 years and 3 months old, 6 months after thiamine supplementation. The images showed near-complete recovery of the lesions after thiamine treatment.

**Figure 3** Cranial MRI of axial section of P7 with *TPK1* deficiency at age of 2 years and 1 month

(a, b, c): The images showed bilateral symmetrical signals in the cerebellar hemispheres, with hypointensity in T1WI phase, while hyperintensity in T2WI and FLAIR phase.

(d, e, f): The images showed bilateral symmetrical patchy signals in the caudate nucleus, putamen, and pulvinar thalamus, with hypointensity in T1WI phase, while hyperintensity in T2WI and FLAIR phase.

**Table 1 Clinical features of seven Chinese patients with thiamine metabolism dysfunction syndrome.**

| Patient | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Age of onset | 4 m | 2 y 5 m | 8.5 m | 1m | 8 m | 1 y 3 m | 1 y |
| Age at first visit | 1 y | 4 y 3 m | 9 m | 1m | 1 y 6m | 1 y 7 m | 2 y 1 m |
| Treatment onset interval | 6 m | 1 y 10 m | 15 d | 2 d | 8 m | 4 m | 1 y 1 m |
| Current age | 2 y 6 m | 6 y 3 m | 11 y | died | 2 y 11 m | 2 y 3 m | 5 y 2 m |
| Sex | F | F | F | F | M | F | F |
| Symptoms at onset | Seizures, developmental regression | General weakness | Dyskinesia, low response | Febrile convulsion | Febrile convulsion | Febrile convulsion | Motor development delay |
| Clinical features | Recurrent epileptic seizures, strabismus, developmental regression, choking sucking, nystagmus, microcephaly, dystonia, dental decay | Episodes of weakness, dysarthria, hypotonia, inability to walk, ptosis, positive Babinski signs bilaterally, sense of numbness | Dyskinesia, opisthotonos, developmental regression, dystonia evolving into hypertonia, low response, equinus | poor intake, poor spirits, hypotonia | Febrile convulsion, confusion, somnipathy, coma, hypertonia of lower limbs, delayed motor | Slightly delayed motor functions, limb shaking | Developmental retrogression, tremble, hypotonia, gait ataxia, paroxysmal muscular weakness |
| Inducing factor | N | Fever | Fever, vaccination | Fever | Fever | Fever | Tumble / Trauma |
| Lactate (0–2 mmol/L) | 2.2 | 1.4 | 1.1 | 3.8-12.7 | 3.5 | 1.9 | 2 |
| Plasma $C_5OH$ (0.2–0.5 μmol/L) | NA | NA | 0.32 | 0.15 | 0.59 | NA | 0.22 |
| Plasma biotin (>200 pg/mL) | NA | NA | NA | NA | 70.09 | NA | NA |
| Urine α-ketoglutarate before /after treatment (26.1–102.9 mmol/mol Cr) | NA / NA | NA / NA | 139 / 130.2 | 229 | 789.1 / 420.3 | 968.4 / 347.9 | 390.3 / 2.23 |
| Brain MRI abnormality | + | + | + | + | + | + | + |
| Diagnosis | Leigh syndrome | Leigh syndrome | Leigh syndrome | Leigh syndrome | Leigh syndrome | Leigh syndrome | Leigh syndrome |
| Main treatment | Biotin 5-10 mg/kg/d, VitB1 10-23 mg/kg/d, topiramate | Biotin 5-10 mg/kg/d, VitB1 8-18 mg/kg/d | Biotin5-10 mg/kg/d, VitB1 10-11 mg/kg/d | Biotin 5-10 mg/kg/d, VitB1 20 mg/kg/d, levetiracetam | VitB1 9-21 mg/kg/d | VitB1 10-21 mg/d | VitB1 7-16 mg/kg/d |
| Attack frequency | Every day | Twice | Four times | Every day | Three times | Once | Four times |
| Follow-up study | less attacks, still unable to raise his head | Normal milestones except occasional sense of numbness in hands and feet | Moderate psychomotor developmental delay | Died at 2 month and a half | Normal intelligence and slight motor developmental delay | Normal intelligence and slight motor developmental delay | Moderate psychomotor developmental delay |
| Phenotype | THMD2 | THMD2 | THMD2 | THMD2 | THMD4 | THMD4 | THMD5 |
| Gene affected | *SLC19A3* | *SLC19A3* | *SLC19A3* | *SLC19A3* | *SLC25A19* | *SLC25A19* | *TPK1* |

Abbreviations: Pt = patient; y = year; m = month; d = day; F= female; M = male; N = none; "+" = positive; "-" = negative; NA = none available; Treatment onset interval = the time from the onset of symptoms to the biotin and thiamine treatment initiation; HI = hearing impairment; $C_5OH$ = hydroxy-isovaleryl carnitine; THMD = thiamine metabolism dysfunction syndrome

**Table 2 Gene mutations detected in seven Chinese patients with thiamine metabolism dysfunction syndrome.**

| Patient | Gene | Region | Nucleotide change | Amino-acid change | Mutation type | Parental derivation | Mutationtaster prediction | PolyPhen-2.0 prediction (score) | SIFT prediction (score) | Conservation | Novel or reported | Accession number |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *SLC19A3* | Exon 5 | 1276_1278delTAC | V426del | Heterozygous | Maternal | Disease causing | - | Deleterious (-10.75) | Yes | Novel | SCV000599769 |
| | | Exon 3 | 265A>C | S89R | Heterozygous | Paternal | Polymorphism | Probably damaging (0.961) | Damaging (0.05) | Yes | Novel | SCV000599770 |
| 2 | *SLC19A3* | Exon 3 | 197T>C | L66P | Heterozygous | Maternal | Disease causing | Probably damaging (0.994) | Damaging (0.04) | Yes | Novel | SCV000599771 |
| | | Exon 3 | 962C>T | A321V | Heterozygous | Paternal | Disease causing | Probably damaging (1.000) | Damaging (0) | Yes | Reported | - |
| 3 | *SLC19A3* | Exon 3 | 265A>C | S89R | Heterozygous | Paternal | Polymorphism | Probably damaging (0.961) | Damaging (0.05) | Yes | Novel | SCV000599770 |
| | | Exon 3 | 850T>C | W284R | Heterozygous | Maternal | Disease causing | Probably damaging (1) | Damaging (0) | Yes | Novel | SCV000599773 |
| 4 | *SLC19A3* | The whole gene deletion | | | Homozygous | Parental | - | - | - | | Novel | SCV001142664 |
| 5 | *SLC25A19* | Exon 4 | 194C>T | A65V | Heterozygous | Maternal | Disease causing | Benign (0.299) | Tolerated (0.09) | Yes | Novel | SCV000599774 |
| | | Exon 5 | 454C>A | P152T | Heterozygous | Paternal | Disease causing | Probably damaging (1) | Tolerated (0.58) | Yes | Novel | SCV000599775 |
| 6 | *SLC25A19* | Exon 6 | 481G>A | A161T | Heterozygous | Paternal | Disease causing | Probably damaging (0.998) | Tolerated (0.16) | Yes | Novel | SCV000599776 |
| | | Exon 6 | 550G>C | A184P | Heterozygous | Maternal | Disease causing | Probably damaging (0.999) | Damaging (0.03) | Yes | Novel | SCV000599777 |
| 7 | *TPK1* | Exon 7 | 395T>C | F132S | Heterozygous | Paternal | Disease causing | Probably damaging (1) | Damaging (0) | Yes | Novel | SCV000599778 |
| | | Intron 7 | c.614-1G>A | - | Splicing | Maternal | - | - | - | Yes | Novel | SCV000599779 |

Note: "-" = None

**Table 3** Amino acids conserved in *SLC19A3* corresponding to codons 66, 89, 284, and 426 (shown in **bold** type).

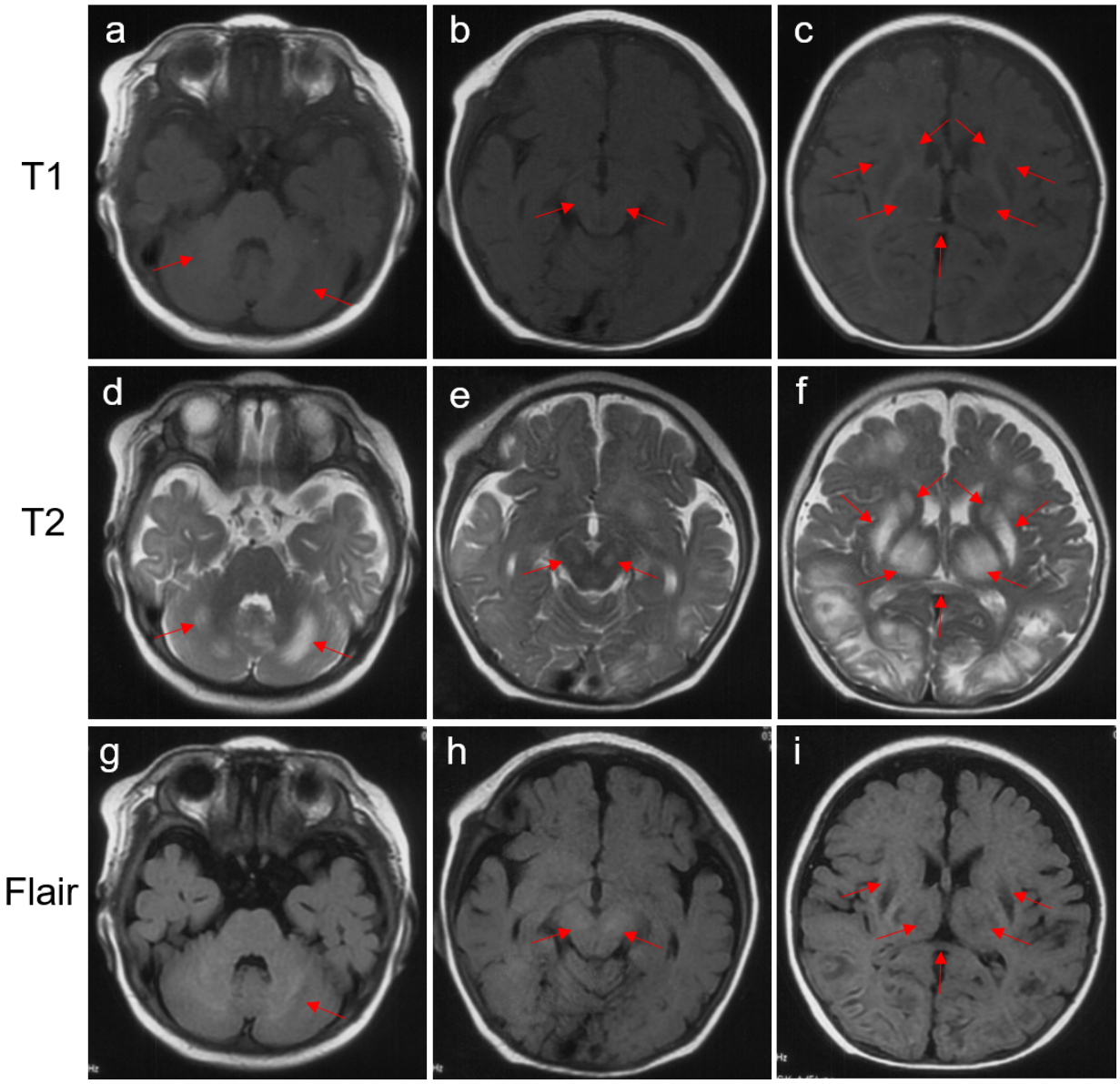
| Organism | Sequence | | | |
|---|---|---|---|---|
| | 66 | 89 | 284 | 426 |
| Human | TLVFVPLL **L** VLYSYTWV | LLLWTIIF **S** IGQLIIVP | GATAFAW **W** LSWYFLRK | PLNLGRQD **V** VIVTMITQ |
| Rhesus | TLVFVPLL **L** VLYSYTWV | LLLWTIIF **S** IGQLIIVP | GATAFAW **W** LSWYFLRK | PLNLGRQD **V** VIVTIITQ |
| Mouse | TLVFVPPL **T** ALYSYTWV | LFLYSTAF **A** VVHLMIVP | GATAFAW **W** LSWYVLHK | PLQLGRQD **V** VIMTMITQ |
| Dog | TLIFVPLL **L** VLYSYTWV | LLLWTIIF **S** IGQLIIVP | GATSFAW **W** LSWYFLRK | PLNLGKQD **V** VIVTMITQ |
| Elephant | TLVFVPFL **L** VLYSYTWV | LLLWTIIF **S** IGQLIIVP | GATSLAW **W** LSWYFLHK | PLNLGRPD **V** VIVTMITQ |
| Chicken | TLLFVPVL **L** ALYSYTWV | LLLWTVIF **S** IGQILLIP | GATALAW **W** LSWYLLKR | DLGLGKSD **V** AVVTLITQ |
| Zebrafish | TLLFVPVL **V** ALYSYTWV | LLVYHLIP **N** FVPTVVVP | GCTALAW **W** ASW-LLTR | DVGLGRSD **V** VVSTIITQ |

Note: bold = the mutant aminio acid

**Table 4** Amino acids conserved in *SLC25A19* (corresponding to codons 65, 152, 161 and 184) and in *TPK1* (corresponding to codon 132)

| Organism | Sequence of *SLC25A19* | | | | Sequence of *TPK1* |
|---|---|---|---|---|---|
| | 65 | 152 | 161 | 184 | 132 |
| Human | EEQLIQRS **A** QLIGHYKA | CACTCACK **P** EGQAAFR | ESRYMTGV **A** HRLTNYV | QLGAYPFI **A** ILTPALG | VSAMIQD **F** RGALGGLTV |
| Rhesus | EEQLIQRS **A** QFIGHYKA | CACTCACK **P** EGQAAFR | ESRYMTGV **A** HRLTNYV | QLGAYPFI **A** ILTPALG | VSAMIQD **F** RGALGGLTV |
| Mouse | EEQLIQKA **A** QFIGHYKA | TGCTCACK **P** EGQAALR | ETKYMTRI **A** ERLNNYI | QLGAYPFI **A** IVTPTLG | VSAMIQD **F** RGGLGGLTV |
| Dog | EEQLIQRG **A** QLIGHYKA | TACTCACK **P** EGQAAFR | ETRYMTVV **A** ERLTKYV | QFGAYPFI **A** ILTPNLG | VSAMIQD **F** RGALGGLTV |
| Elephant | EEQLIQRG **A** QLIGHYKA | CACTCACK **P** EGQAAFR | ETRYMTVV **A** DRLTKYI | QFGAYPFI **A** ILTPNLG | VSAMTQD **F** RGALGGLTV |
| Chicken | ======== = ======== | ======== = ======= | ======== = ======= | QFGAYPFV **A** IVTPTLG | VSAMIQD **F** RGGLGGLTV |
| Zebrafish | EETLICRT **A** QWLGWYKG | C--CTACK **P** EGQAAFR | ESRLMTGI **A** HRLNRYI | QLGAYPFV **A** VLTPTLG | VTAMTQD **F** RGGLGGLTV |

Note: bold = the mutant aminio acid


T1
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