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THIAMINE DEFICIENCY IN CHILDHOOD WITH ATTENTION TO GENETIC CAUSES: SURVIVAL AND OUTCOME PREDICTORS.

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Key Words: Biotin responsive basal ganglia disease, Leigh syndrome, *SLC19A3*, *SLC25A19*, *TPK1*, thiamine, biotin, Wernicke encephalopathy, beriberi.

Abbreviations

BCODC: branched chain 2-oxo acid dehydrogenase complex.

BTRBGD: biotin thiamine responsive basal ganglia disease

CSF: Cerebrospinal fluid

LS: Leigh Syndrome

MRI: Magnetic resonance imaging

OGDHC: oxoglutarate dehydrogenase complex

OXPHOS: Oxidative phosphorylation.

PDHc: pyruvate dehydrogenase complex

SEM: Standard error of the mean

ThTR2: thiamine transporter-2

ABSTRACT

Primary and secondary conditions leading to thiamine deficiency have overlapping features in children, presenting with acute episodes of encephalopathy, bilateral symmetric brain lesions and high excretion of organic acids that are specific of

thiamine-dependent mitochondrial enzymes, mainly lactate, alpha-ketoglutarate and branched chain keto-acids. Undiagnosed and untreated thiamine deficiencies are often fatal or lead to severe sequelae. Herein we describe the clinical and genetic characterization of 79 patients with inherited thiamine defects causing encephalopathy in childhood, identifying outcome predictors in patients with pathogenic *SLC19A3* variants, the most common genetic etiology. We propose diagnostic criteria that will aid clinicians to establish a faster and accurate diagnosis so that early vitamin supplementation is considered.

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Keywords: *SLC19A3*, *SLC25A19*, *TPK1*, thiamine, biotin, striatal necrosis, Leigh syndrome, thiamine encephalopathy, mitochondrial disease.

Introduction

Thiamine or vitamin B1 is a critical cofactor involved in energy metabolism and in the synthesis of nucleic acids, antioxidants, lipids and neurotransmitters.^{1,2} Thiamine is a

water-soluble essential nutrient obtained from cereals, meat, eggs, legumes and vegetables. In the absence of adequate thiamine intake, limited tissue storage may be depleted in 4 to 6 weeks.³ Thiamine requires specific transporters for the absorption in the small intestine and for cellular and mitochondrial uptake (thiamine transporter-1, encoded by *SLC19A2*, thiamine transporter-2, encoded by *SLC19A3*, and mitochondrial thiamine diphosphate carrier, encoded by *SLC25A19*). Within the cellular compartment, thiamine is converted into thiamine diphosphate by thiamine phosphokinase (*TPK1*), the metabolically active form of thiamine, which acts as a cofactor of several thiamine-dependent enzymes in the cytosol, peroxisomes and mitochondria (**Figure 1**). Specifically, in the mitochondria TDP acts as a cofactor of the PDHc (pyruvate dehydrogenase complex), OGDHC (oxoglutarate dehydrogenase complex) and BCODC (branched chain 2-oxo acid dehydrogenase complex).

Infantile beriberi and Wernicke encephalopathy are rare life-threatening and reversible causes of secondary thiamine deficiency that still occur in vulnerable populations.⁴

Infantile beriberi presents in infants breastfed by mothers with inadequate intake of thiamine⁵ or receiving low thiamine-content formula⁶, whereas Wernicke encephalopathy is described in sick children that undergo medical or surgical procedures such as gastro-intestinal resections, parenteral nutrition, chemotherapy, etc.⁷

In recent years, genetic defects in thiamine transport and metabolism have been described in childhood, with overlapping clinical, biochemical and radiological features to those observed in secondary forms, and a good response to vitamin supplementation.

Well-defined clinical phenotypes have been recognized in the following defects:⁸ (1) *SLC19A2* (thiamine transporter-1) causes Roger's syndrome or thiamine responsive megaloblastic anemia (OMIM 249270); *SLC19A3* (thiamine transporter-2, ThTR2) is responsible for biotin thiamine responsive basal ganglia disease (BTRBGD) (OMIM

607483); Leigh syndrome (LS); infantile spasms with lactic acidosis; and Wernicke-like encephalopathy; (3) *TPK1* (thiamine phosphokinase) causes LS (OMIM 614458); and finally (4) *SLC25A19* (mitochondrial thiamine pyrophosphate carrier) produces Amish microcephaly (OMIM 607196) and bilateral striatal degeneration and progressive polyneuropathy (OMIM 613710). Interestingly, three of these genotypes (*SLC19A3*, *TPK1* and *SLC25A19*) present with acute encephalopathy, basal ganglia lesions and lactic acid accumulation due to brain energy failures.⁹⁻¹² These clinical and radiological manifestations are indistinguishable from LS, a severe neurological disorder of brain energy production, caused by more than 88 genetic variants.^{13,14}

In this study we provide a global overview of the numerous genetic and acquired etiologies of thiamine deficiency in childhood, with specific attention to inherited defects of thiamine transport and metabolism. We analyze the clinical features and long-term outcomes in a multi-ethnic cohort of seventy-nine *SLC19A3*, *SLC25A19* and *TPK1* patients, evaluate how thiamine/biotin treatment modifies the natural history of *SLC19A3* patients, and identify the clinical parameters that may help predict neurological outcome and guide further therapeutic interventions. We propose fundamental features to suspect inherited thiamine defects against external or secondary causes of thiamine deficiency, and suggest diagnostic criteria that will help clinicians to establish faster and accurate diagnosis so that early vitamin supplementation is considered.

Methods

Study design. We conducted a multicenter cohort study by reviewing data from patients with inherited defects in thiamine transport and metabolism. We invited forty-four investigators that had published patients with inherited thiamine defects and/or patients

with Leigh syndrome and mitochondrial disorders in PubMed. In total, twenty-one investigators accepted to participate, seventeen centers did not have patients to include and lastly, six colleagues did not answer or refused to participate in the study.

A systematic analysis in MEDLINE (through PubMed) was performed to search for secondary causes of thiamine deficiency. We included the following keywords: #1 beriberi, #2 Wernicke encephalopathy and #3 secondary thiamine deficiency, from January 2010 to February 2017. We analyzed predisposing factors, consanguinity, clinical, biochemical and radiological features, mortality, treatment, recovery, neurological and radiological sequelae.

Study population. We included patients with two pathogenic variants of the *SLC19A3*, *TPK1*, and *SLC25A19* genes. Patients with *SLC19A2* mutations were excluded from the study, as they did not have significant involvement of the central nervous system. The responsible clinician at each collaborating center collected data via a questionnaire that consisted of 131 items including: demographic data, family and perinatal history, genetic defects, gene-related phenotype, early developmental milestones, age of disease onset, triggering events, clinical neuroimaging and biochemical data at disease onset, thiamine and biotin supplementation and follow-up. In surviving patients presenting with dystonia, disability was evaluated using part b of the Burke-Fahn-Marsden scale (BFMDS). This questionnaire evaluates the dystonic patient's ability to perform everyday activities, and has been used previously in *SLC19A3* patients.¹⁵ One hundred and twenty-nine patients with nuclear encoded complex I deficiency and 324 patients with pyruvate dehydrogenase complex (PDHc) deficiency were collected through a review of the research literature in order to perform a comparative survival analysis. The time of death of nuclear encoded complex I patients was quantified according to methods described by **Ortigoza-Escobar et al., 2016.**¹⁶ References of patients

collected with PDHc deficiency included for comparative survival analysis appear in supplemental material.

Standard protocol approvals, registration and patient consent. This study was approved by the Ethics Committee of the Hospital Sant Joan de Déu, Barcelona, Spain. Informed consent was obtained from all patients.

Statistical analysis. Statistical analyses were performed using IBM SPSS Statistics 23 software (IBM Corp., Armonk, NY, USA). The quantitative variables were reported either in terms of the normal distribution mean, standard error of the mean (SE) and the range; or in terms of the median and interquartile range (IQR). The Mann-Whitney U test was applied to evaluate differences in numerical variables between groups. The chi-square test and Fisher's exact test were used to test the association between categorical variables. Multiple logistic regression analysis was performed to further investigate the relationship between the binary response variable and potential predictors of survival. The Kaplan-Meier survival analysis was used to compare the survival rates of the *SLC19A3*-deficient patients, patients with PDHc and nuclear-encoded complex I-deficient LS. Differences in survival between the groups were evaluated using the log rank test. All statistical tests were two-sided and performed at a 0.05 significance level.

Results

1. Inherited thiamine defects

We identified 70 patients with *SLC19A3* disease, 4 patients with *TPK1* disease and 5 patients with *SLC25A19* disease. The patients were diagnosed at twenty-one centers: UK (n=4), USA, Germany and Finland (n=3), Saudi Arabia (n=2), The Netherlands, Spain, Israel, France and Sweden (n=1). Genotypes were established in all patients, including P76 who had a similar disease course to his sibling with *TPK1* deficiency and

in whom the same mutations were confirmed using residual DNA. Complete clinical data sets were available in 65/70 patients with the *SLC19A3* mutation and in all patients with *SLC25A19* and *TPK1* mutations. Magnetic resonance imaging (MRI) data were available in all except seven *SLC19A3* patients who were diagnosed post-mortem (P39 to P45). None of the patients were found to have additional clinical or biochemical abnormalities suggestive of other genetic diseases.

1.1. *SLC19A3*

The 70 patients with *SLC19A3* deficiency (mean age at assessment $\pm$ SE: 9.5 $\pm$ 0.9 years, range 1 month – 40 years old, 36 males, 51%) were born between 1975 and 2015. Of these, 63 (90%) had been previously reported. Consanguinity was reported in 51 (73%) patients and 44 (62%) had other affected family members. Arabs formed the largest ethnic group (58/70, 82%: Saudi Arabian n=41, Moroccan n=11, Iraqi n=3, Kurdish n=2, Kuwaiti n=1), followed by White European (10/70, 14%: Spanish n=3, Portuguese n=2, German n=2, Finnish n=2, Hispanic n=1), and African/Afro-Caribbean (2/70, 2.8%) (Supplementary Table 1).

Clinical phenotype

Supplementary Table 1 summarizes the demographic, genetic, clinical and radiologic features in the entire patient cohort. The frequency of the main clinical features appears in Figure 2. Fetal distress was noted in P2, P29 and P61 and acute presentation during the newborn period (around 4 weeks of age in all cases) was reported in 9 (12%) patients. In the vast majority the developmental milestones were average, except in 7 cases (P28, P29, P53, P57, P60, P61 and P66)

The median age at disease onset was 3 years, the range was 1 month – 34 years and the IQR was 1 – 2.8 years. The trigger events (39/70 patients, 55%) were viral (n=30) or

bacterial (n=4) infection, trauma (n=3), profuse exercise and vaccination (n=1, each).

Fifteen (21%) patients were classified as LS and fifty-three (75%) as BTRBGD due to their positive responses to thiamine/biotin treatment. Twins (P35 and P36) with a positive family history (siblings of P34) were identified before the onset of symptoms.

Twenty-six patients experienced more than one encephalopathic episode before the initiation of vitamin supplementation (2 episodes n=12, 3 episodes n=7, 4 episodes n=4 and 5 or more episodes n=3 [P32, P33 and P49]). Most episodes of neurological deterioration were triggered by infection or stress. Intensive care was required in 5 of the patients (P1, P3, P48, P68, and P69).

A minority of patients (10/62, 16%) had an insidious onset of symptoms characterized by psychomotor regression, hyperactivity and attention deficit, unsteady gait, toe walking or stiffness of the limbs.

Systemic features of mitochondrial disease (cardiomyopathy, cardiac conduction defects, renal tubulopathy or facial dysmorphism) were absent in our series. P4 developed a steroid-sensitive nephrotic syndrome.

Seizures were classified according to the International League Against Epilepsy (ILAE) as follows: generalized seizures (n=31), focal seizures (n=6, including two patients [P30 and P31] with *epilepsia partialis continua*), epileptic spasms (P64, n=1) and West syndrome (P48, n=1). Thiamine and biotin treatment controlled seizures in patients effectively, except in P38, P53 and P59, who also received antiepileptic drugs, and P49, P61 and P64, who developed drug resistant epilepsy.

Ancillary testing

Few patients showed increased CSF lactate (5/29 patients, mean $\pm$ SE: 3.7 $\pm$ 1 mmol/L, range 2.1-7.1 mmol/L; NV<2), increased blood lactate (20/40, mean $\pm$ SE: 4.1 $\pm$ 0.4 mmol/L, range 2.1-8.6 mmol/L, NV<2), metabolic acidosis (6/36) and increased blood alanine (4/15, mean $\pm$ SE: 666 $\pm$ 129 μ mol/L, range 450-1037 μ mol/L, NV<439). The lactate/pyruvate ratio was below the normal limit in two patients. Increased blood lactate levels were negatively correlated with the age of onset in the *SLC19A3* patients (p <0.001).

Abnormal organic acid profiles were recorded in 8/50 patients, with high excretion of isobutyric, 2-OH-isovaleric and 2,4-di-OH-butyric (P1), alpha-ketoglutaric (P2), lactic (P34 and P52), 3-OH-butyric (P37), 2-OH-glutaric, glutaric, succinic and 2 keta adipic acid (P41) and 4-OH-phenyllactic (P46).

MRI and MRS were available in 61/70 (87%) and 43/70 (61%) patients, respectively. All symptomatic patients had lesions at onset, involving bilateral caudate (n=55), putamen (n=57), cortico-subcortical areas of the cerebral hemispheres (n=40), ventromedial region of the thalamus (n=38), cerebellum (n=23), brainstem (n=14), periaqueductal region (n=12), spinal cord (n=11) and the globus pallidus (n=6) (**Figures 2 and 3**). Acute MRIs indicated swelling and chronic MRIs indicated volume loss and necrotic changes. MRS detected a lactate peak in 55% (24/43) patients within the affected areas. Stroke-like lesions or mammillary body lesions were not identified.

Statistical analysis showed that deceased patients had more frequent involvement of the globus pallidus and brainstem than surviving patients (3/15 vs. 3/55, Mann-Whitney U test, p=0.001; and 4/15 vs. 10/55, Mann-Whitney U test p=0.009, respectively).

OXPPOS activity was normal in the muscle and skin biopsies of 6 patients, with the exception of P41 who showed 56% of complex IV activity in fibroblasts. None of the patients had ragged red fibers.

Treatment and outcome

Fifty-one patients received vitamin supplementation during the acute encephalopathic episode. The time from disease onset to vitamin initiation was very broad (median 14 days, IQR: 4–180 days). Forty-four patients had a significant clinical recovery within hours or days of vitamin initiation: they regained alertness, improved feeding, had a better control of seizures and gradually recovered previously acquired milestones. Four more patients showed a mild improvement and three patients did not improve at all.

Fifty-five patients were alive at the time of recruitment (mean follow-up 5.2 ± 0.7 years, range 2 weeks–22 years). All of them received thiamine (thiamine hydrochloride, mean dose 20 mg/kg/day, range 5 to 55) and forty-seven received biotin (mean dose 5 mg/kg/day, range 1 to 30). Both vitamins were administered orally in most patients, although some patients received intravenous supplementation in the acute episode. The neurological examination was normal in twenty-six patients at the time of assessment and they were symptom-free, whereas twenty-seven had developed some neurological sequelae (**Supplementary Table 1**). No further decompensating episodes of encephalopathy, dystonia or other neurological symptoms were recorded after vitamin supplementation in these patients, except for P61 who received inadequate vitamin doses. Additionally, blood alanine levels and the organic acid profiles in urine were normal in patients receiving vitamin supplementation. Only P52 had slightly elevated blood lactate (2.3 mmol/L). The twins (P35 and P36) who were treated pre-

symptomatically with thiamine alone were symptom-free at the time of assessment (5 years).

Fifteen patients (21%) died, the majority of them from central respiratory failure (6.1 ± 1.9 years at death, range: 4 weeks - 20 years). Deceased patients were younger at onset compared to surviving patients (mean age $\pm$ SE 2.4 ± 1.1 years vs. 5.4 ± 0.9 years, Mann–Whitney U test, $p=0.005$). Among the fifteen deceased patients, four had received vitamin supplementation (P1, P30, P41 and P49).

Disability score

The BFMDS questionnaire was administered to 34 *SLC19A3* patients with dystonia (9.8 ± 1.8 points (mean $\pm$ SE), range 0–30 points). Higher BFMDS scores were identified in patients who had a previous history of developmental delay (19.5 ± 4.1 vs. 7.7 ± 1.7 , Mann–Whitney U test, $p=0.017$) and in patients with disease onset before 6 months of age (23.7 ± 2.8 vs. 7.9 ± 1.7 , Mann–Whitney U test, $p=0.01$). A positive and almost significant correlation was observed between the BFMDS scores and the time from disease onset to thiamine initiation (Pearson correlation, $r=0.340$; $p=0.053$) (**Figure 4D**).

Survival analysis

In the Kaplan-Meier analysis (**Figure 4**), treated *SLC19A3* patients had a longer mean survival length than non-treated patients (A) (28.99 vs. 17.23 years, log rank test, $p<0.0001$). Additionally, mean survival length was longer in homozygous c.1264A>G *SLC19A3* patients than in patients with other mutations (B) (29.88 vs. 15.52 years, log rank test, $p<0.0001$). Homozygous c.1264A>G patients were comparable to patients with other mutations with respects to age at disease onset and age at treatment initiation.

However, a significant difference was observed between both groups in the number of

treated patients (39/44 (88%) treated c.1264A>G patients versus 13/22 (59%) treated patients with other mutations, $p=0.006$). Mean survival length was longer in the 70 *SLC19A3* patients than in 129 patients with nuclear-encoded complex I deficiency (C) (28.0 vs. 11.5 years, log rank test, $p<0.001$). Similar results were obtained when the *SLC19A3* and Complex I patients were divided into two age groups: those with disease onset before 6 months (11.3 vs. 2.9 years, Log rank test, $p=0.004$) and those with a later onset (log rank test, 33.3 vs. 22.6 years, $p=0.003$). No significant differences were observed in the mean survival length between 70 *SLC19A3* and 324 PDHc patients in both age groups. However, an almost significant difference was observed when selecting male PDHA1 patients.

***SLC19A3* gene mutations**

We identified 13 *SLC19A3* pathogenic mutations (**Supplementary Table 1, Figure 4**) in the 70 patients. Five of these mutations were novel (c.91T>C, c.157A>G, c.503_505delCGT, c.516_delC and c.833T>C). Fifty-nine patients were homozygous for the following missense mutations: c.1264A>G ($n=, 47$), c.20C>A ($n=7$), c.157A>G ($n=3$), c.68G>T ($n=1$) and c.541T>C ($n=1$). Eleven patients were compound heterozygotes. The most frequently occurring mutation in our cohort, c.1264A>G, was present in patients with Arab ethnic backgrounds, including Saudi Arabian, Moroccan, Kurdish and Kuwaiti patients. The next most common mutation, c.20C>A, occurred exclusively in subjects from the province of Al Hoceima in Northern Morocco ($n=7$, 3 pedigrees).⁹ The splice mutation c.980-14A>G was observed in 5 compound heterozygote individuals, all of them of White European origin.

1.2. *SLC25A19*

We recruited 4 consanguineous Arabic patients from Israel (homozygous for c.373G>A)¹² and a new White European German patient diagnosed by our group (P75).

The phenotype of this girl, age 21 years, was similar to previously reported cases (**Supplementary Table 1**). The symptoms were triggered by febrile illness between 20 months and 6.5 years of age, and consisted of acute encephalopathy, dysarthria, and episodic flaccid weakness. They had elevated levels of lactate in the CSF at onset (2.9–4.2 mmol/L, NV<2 mmol/L) but normal systemic mitochondrial biomarkers. MRI showed T2 hyperintensity and necrosis in the caudate and putamen in all patients. Additionally, P74 had T2 hyperintensity (cavitated lesion) in the medial thalamus.

Thiamine treatment (400 – 600 mg/day) administered three years after onset led to substantial improvement in peripheral neuropathy and gait in P71 and P72, whilst P74 and P73, treated 9 and 12 years later, respectively, continued to have significant ambulatory impairment. All patients are currently alive. They have mild to severe bilateral *pes equinus*, axonal polyneuropathy and dystonia. The disease severity was clearly milder in P71, P72 and P75, supporting the efficacy of thiamine treatment in preventing further disease progression.

Interestingly, P73 stopped thiamine treatment due to an apparent lack of benefit. Four years later, a severe episode of flaccid paralysis occurred with fever, and intravenous thiamine (1.500 mg) led to clinical recovery within 24 hours.

1.3. *TPK1*

Four *TPK1* patients (P76, P77 and P79, previously reported),^{17,18} suffered from LS triggered by febrile illness between one month and 2.5 years of age (**Supplementary Table 1**). Brain lesions developed in the cerebellum and dentate nuclei (n=4), striatum (n=3), thalamus, globus pallidus (n=2), brainstem and spinal cord (n=1). P76 showed

lactic acidosis (3.0 mmol/L, NV < 1.77 mmol/L) whilst none had increased lactic acid in the CSF. Increased excretion of organic acids was recorded in 3 out of 4 patients: lactic acid (P76), glutaric acid (P77) and mildly increased alpha-ketoglutaric acid and dicarboxylic acid (P79). P76 and P78 who did not receive thiamine supplementation died at the age of 29 and 6 months, respectively. P77 and P79 are currently 4 and 7 years old, respectively. P77 receives a combination of thiamine (15 mg/kg/d), biotin (1 mg/kg/d, P77) and ketogenic diet and P79 receives thiamine (500 mg/day) alone. Both patients show severe neurologic sequelae, with spasticity, hypotonia, dystonia, developmental delay and high scores on the BFMDS (27 and 14, respectively).

Secondary thiamine deficiency

A total of 153 patients (beriberi: N=88; Wernicke encephalopathy: N=65) were collected, age between 2 weeks and 17 years of life.^{4, 19-42} A summary of the main characteristic features collected in our database is provided in **Figure 6**. Predisposing factors were reported in 100% of cases. Systemic, cardiovascular and respiratory symptoms were recorded in all patients with beriberi and a few cases with Wernicke encephalopathy. The majority of cases showed increased blood 53/55 (96%) and CSF lactate, 12/13 (92%) and metabolic acidosis: 17/34 (50%). Low blood thiamine levels were detected in 100% of cases that were analyzed. The thalamus, periaqueductal region and mammillary bodies were more frequently involved, as opposite to patients with inherited thiamine defects, who showed more frequent alterations in the caudate and putamen. Clinical improvement after thiamine supplementation was constant, and less than 20% showed mortality or neurological sequel.

Discussion

Thiamine diphosphate, the metabolically active form of thiamine, is essential for energy production in the central nervous system. In brain regions with high metabolic demands, thiamine deficiency due to exogenous (nutritional) or endogenous (genetic) defects can trigger a metabolic crisis, and Wernicke's encephalopathy is a model of cerebral thiamine deficiency.⁴³ Primary and secondary conditions leading to thiamine deficiency have overlapping features in children, both presenting with acute episodes of encephalopathy, bilateral symmetric brain lesions, and biomarkers of mitochondrial dysfunction, such as lactic acid accumulation in different tissues and high excretion of organic acids. In both scenarios, early thiamine supplementation may lead to clinical recovery within a few hours or days, and brain lesions may be reversible on MRI.

In our international study group of 79 patients with inherited thiamine defects, the vast majority of children were born to consanguineous patients, they presented between the age of 1 and 6 years in the context of a febrile illness, with acute/recurrent encephalopathy, basal ganglia lesions, dystonia, hypotonia, spasticity, ataxia and seizures. Lactic acid accumulation in the CNS was identified by MRS in half of the *SLC19A3* patients, and in all *SLC25A19* cases by CSF analysis, suggesting brain energy failure, as in other cases of Leigh encephalopathy.¹³ Also, abnormal organic acids were identified in a few *SLC19A3* and *TPK1* cases. These included organic acids that were specific of thiamine dependent mitochondrial enzymes, such as lactic acid (PDHc), alpha-ketoglutarate (OGDHC) and branched chain keto-acids (BCODC), and other non-specific organic acids caused by generalized mitochondrial dysfunction. These biomarkers did not predict outcome in our series.

Fundamental features to suspect primary thiamine defects against secondary etiologies were the frequency of consanguineous families, the absence of predisposing factors for

beriberi and Wernicke encephalopathy, the lack of cardiovascular or respiratory features, and the sparing of mammillary bodies on MRI (**Figure 6**).

A characteristic feature that distinguished patients with mutation in *SLC25A19* from the other genetic defects was the presence of peripheral neuropathy. This phenotype was initially described in Spiegel et al., 2009,¹² and to our knowledge no further patients have been reported since then. We identified a novel missense variant in the *SLC25A19* gene in a 20-year-old woman with striatal necrosis and peripheral neuropathy, thus providing clinical evidence to the recognition of this phenotype. In contrast to *SLC19A3* and *SLC25A19*, patients with mutation in *TPK1* had an earlier onset of symptoms, they showed variable response to thiamine supplementation, and developed a more severe phenotype with higher morbidity and mortality.

Based on the frequency of the main clinical and radiological features, and on specific biomarkers, we propose diagnostic criteria for the three known inherited thiamine defects with prominent neurologic involvement (**Table 1**). Total thiamine levels in blood are reduced in patients with secondary deficiencies, but are normal in genetic conditions⁴⁴⁻⁴⁶, in which the quantification of thiamine isoforms, either in blood, CSF, fibroblasts or muscle cells, is critical for the diagnosis.^{13,47}

More than one third of *SLC19A3* patients had several recurrent encephalopathic episodes before vitamin supplementation. Most of them were born after the first description of the disease by Ozand et al. in 1998,⁴⁸ suggesting that this is an under diagnosed disorder. Thiamine and biotin supplementation led to prompt and significant clinical recovery in most *SLC19A3* patients. Survival analyses showed that the mean survival length was longer in patients who received thiamine and biotin compared to non-treated patients. No further episodes of encephalopathy, dystonia or other

neurological disturbances were noted after vitamin supplementation. Additionally, patients had effective control of seizures without the need of antiepileptic drugs and nonspecific biomarkers of mitochondrial dysfunction remained within normal limits. More importantly, half of treated patients had no disability at all, and neurological examination was normal at the time of assessment. A positive correlation was observed between the disability scores and the time spent between disease onset and thiamine initiation, meaning that the earlier the therapeutic intervention, the lower the sequel observed, although this data did not reach statistical significance. It was likely that the deceased patients with severe disabilities were missing relevant data, representing an important bias against a significant correlation in this analysis.

We identified clinical parameters that may help predict neurological outcomes before the initiation of vitamin supplementation. Patients with symptoms presenting within the first 6 months of life had a shorter survival curve and higher scores on the disability scale. Moreover, the distributions of brain lesions in the deceased *SLC19A3* patients were more diffuse, with a significantly higher involvement of the globus pallidus and brainstem. This is in accordance with previous reports that indicate a subgroup of patients with thiamine transporter 2 deficiency presenting with fatal infantile Leigh syndrome, who died very early in the disease course, or were left with severe neurological sequel and extensive brain injury despite the initiation of vitamin treatment.^{9,49} In our cohort, four children died despite vitamin supplementation. Pre-treatment predictors of poor responses in these patients were extensive brain involvement (P49), early onset of the disease (P41) or the co-occurrence of septicemia in a child receiving intensive care (P1).

Based on these data, we believe that patients presenting with Leigh syndrome should be treated with a vitamin cocktail including thiamine and biotin, as the phenotype of

inherited thiamine defects may be clinically indistinguishable from other genetic disorders leading to Leigh encephalopathy. However, therapeutic interventions should be individualized in those cases presenting clinical predictors of poor neurological recovery.

SLC19A3 patients showed longer survival length than patients with complex I deficiency, with more than 60% of the *SLC19A3* patients surviving at 20 years of age. Previous studies have demonstrated poor survival rates in LS patients.⁵⁰⁻⁵⁵ Factors associated with poor prognosis in LS patients were the onset of symptoms within the first 6 months, the presence of cardiomyopathy⁵⁶ and brainstem involvement.⁵⁰ As opposite to these features, *SLC19A3* patients in our series had a median age at disease onset of 3 years, they did not show cardiac involvement, and brain stem lesions were observed in a minority of cases. More importantly, thiamine supplementation prolonged survival in *SLC19A3* patients, whereas no effective treatment is available for complex I deficiency patients. We didn't observe differences on survival between *SLC19A3* and PDHc patients; however, an almost significant difference was observed when selecting male patients with PDHA1 deficiency, which are known to have a poorer survival than females. PDHc patients benefit from ketogenic diet⁵⁷ and thiamine supplementation,⁵⁸ which reduce neurological features and the frequency of hospitalizations.

A high proportion of *SLC19A3* patients in our cohort were homozygous for the missense variant c.1264A>G, all of them belonging to the Arab ethnic group. These patients had a longer survival than patients with other mutations. The higher proportion of patients receiving thiamine and biotin in the group of homozygous c.1264A>G patients compared to patients with other mutations may account for these differences. In line with this observation, transfection studies demonstrated that the c.1264A>G

mutation led to a protein with null-transport activity,⁵⁹ which is difficult to correlate with a more benign phenotype.

Among patients from European countries, we identified four compound heterozygous cases with c.74dupT/c.980-14A>G that had in common a late disease onset, three of them in adolescence and adulthood, with an excellent response to thiamine.^{60,61} Likewise, a recently described patient with the same mutations presented an adult-onset subacute leukoencephalopathy with an effective response to thiamine.⁶²

Our results confirm previous reports on a characteristic pattern of brain injury in inherited thiamine defects^{9-12, 17, 19, 45, 60, 61, 63} that will help in the early recognition and differential diagnosis. The majority of *SLC19A3* patients showed striatal lesions in combination with other affected brain areas, including cortico–subcortical regions of the cerebral hemispheres, ventromedial thalamic nuclei, cerebellum, brainstem, periaqueductal region, spinal cord and globus pallidus, in order of decreasing frequency. As previously mentioned, deceased patients had a greater involvement of the brain stem and globus pallidum. Lesions in the respiratory centers, located in the medulla oblongata and pons, may be responsible for acute respiratory failure, the most frequent cause of death in this cohort, as in other LS patients. Moreover, bilateral lesions in the globus pallidus were also related to severe dystonia and poor prognosis in patients with glutaric aciduria type I and in carbon monoxide intoxication.^{64,65} Isolated striatal necrosis was a common feature of *SLC25A19* patients. In contrast, TPK1 patients showed basal ganglia lesions in combination with involvement of the cerebellum and dentate nuclei.

The small size of the sample was an important limitation for this study. Also, recruitment was restricted to published cases and individual centers, leading to a

possible selection bias toward more severe presentations. These are common limitations for research in rare diseases, where the limited number of patients hampers the recognition of the full spectrum of severities. Also, a more accurate assessment on neurodevelopment should be warranted in future studies.

In summary, this international study describes the natural history of seventy-nine patients with inherited defects in thiamine transport and metabolism. We confirm that thiamine defects manifest with acute brain injury in the first decade of life in the vast majority of patients. We demonstrate how vitamin supplementation modifies the survival curve of *SLC19A3* patients, and identify statistically significant predictors of neurological outcome that may guide clinicians in further therapeutic interventions. Our study indicates a better prognosis than other causes of Leigh syndrome, encouraging clinicians to suspect the disease and to make an early diagnosis and accurately prescribe treatment. We also contribute with diagnostic criteria for inherited thiamine defects and help differentiate them from secondary causes of thiamine deficiency.

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Author Contributions

The following authors contributed to the study concept and design (JDOE, BPD), analysis of data (JDOE, MA, MML, ND, RS, IFdeC, MG, RA, MN, PRP, BT and BPD), and drafting of the manuscript and figures (JDOE, BPD, MML, RA, PRP). Authors who participated in data acquisition were included into the Thiamine Deficiency Study Group (JL, AH, FD, PI, TL, LU, AV, JLF, RMcF, RT, SS, EW, RD, YN, SB, ES, JRF, MRZ, EV, AD and LM). All authors participated in editing and approving of the manuscript.

Potential Conflicts of Interest. None.

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Figures

Figure 1. Schematic layout of the thiamine transport and metabolism. There are four known forms of thiamine in humans, free non-phosphorylated thiamine (free-T, purple balls) and its phosphate esters: thiamine monophosphate (TMP, green balls), thiamine diphosphate (TDP, red balls), and thiamine triphosphate (TTP, non-represented). Although at high concentrations, thiamine absorption is via passive diffusion, thiamine is absorbed in the small and the large intestine and transported across the blood-brain barrier using several well-known transporters: *SLC19A1* (folate transporter) *SLC19A2* (thiamine transporter-1), *SLC19A3* (thiamine transporter-2), *SLC44A4* (human TDP transporter), *SLC22A1* (OCT1, organic cation transporter 1) and *SLC35F3*. At that point intracellular free-T is converted to TDP, which is the metabolically active form of the vitamin, by *TPK1* (thiamine pyrophosphokinase) and transported inside the mitochondria by the *SLC25A19* (mitochondrial TDP transporter). Human TDP-dependent enzymes comprise TK (transketolase), HACL1 (2-hydroxyacyl-CoA lyase 1), PDHc (pyruvate dehydrogenase complex), OGDHC (oxoglutarate dehydrogenase complex) and BCODC (branched chain 2-oxo acid dehydrogenase complex).

Figure 2. Major clinical features and neuroimaging results in 70 SLC19A3-

deficient patients. A. Encephalopathy defined as lethargy, irritability, agitation, vomiting, continuous crying, coma leading to ventilatory support, etc. Status dystonicus defined as the need of specific management, such as admission to pediatric intensive care unit, sedation and ventilatory support, benzodiazepines, baclofen, clonidine, anticholinergic, chloral hydrate, DBS, etc. Spasticity includes hyperreflexia and signs of Babinski reflex. Liver disease defined as increased liver enzymes, liver failure or hepatomegaly. The number of patients is plotted on the x-axis and the symptoms and signs are plotted on the y-axis. **B.** Most patients presented a characteristic radiological pattern with hyperintensities in the caudate, putamen, ventromedial region of thalamus and diffuse cortico-subcortical areas. Statistical analysis indicated that deceased patients had more frequent involvement of the globus pallidus (3/15, 20%, vs. 3/55, 5%, $p=0,001$) and brainstem (4/15, 26% vs. 10/55, 18%, $p=0,009$) than surviving patients.

Figure 3. MRI patterns in patients with secondary and inherited thiamine defects.

Wernicke encephalopathy. Axial T2W, sagittal and coronal FLAIR images show bilateral symmetric involvement of dorsal medial thalamus, periaqueductal gray matter, mammillary bodies (white arrow) and patchy cortical and subcortical hyperintensities. SLC19A3. Axial and coronal T2W images show bilateral symmetric involvement of the putamen and thalamus along with patchy cortical and subcortical hyperintensities. SLC25A19. Axial T2W and T1W images show cystic necrosis of the caudate and putamen. TPK1. Axial and coronal T2W SE images show involvement of the posterior putamen and dentate nuclei (gray arrow).

Figure 4. The figure shows Kaplan-Meier survival curves (A, B, C) and the correlation between the Burke-Fahn-Marsden Disability Scale and the time elapsed between disease onset and thiamine supplementation in SLC19A3 patients

(D). **A.** Comparison between treated (n=51) vs. untreated (n=19) *SLC19A3*-deficient patients; log rank test $p<0.0001$. **B.** Comparison between c.1264A>G homozygous mutation (n=44, 39 (88%) treated patients) vs. other mutations (n=22, 13 (59%) treated patients) in *SLC19A3*-deficient patients; log rank test $p<0.0001$. **C.** Comparison between *SLC19A3* patients (n=70), nuclear-encoded complex I deficient Leigh syndrome (n=129) and male PDHc deficient patients due to PDHA1 deficiency (n=145). When comparing *SLC19A3* and nuclear-encoded complex I deficient Leigh syndrome, differences reach statistical significance (log rank test $p<0.001$). When comparing *SLC19A3* and male PDHc patients, differences did not reach statistically significance (log rank test $p=0.06$). **D.** Correlation between the BFMDS (y-axis) and the time elapsed between disease onset and thiamine supplementation (x-axis, days, log-scale) in *SLC19A3* patients ($r=0.34$, $p=0.053$).

Figure 5. Pathogenic mutations in the human thiamine transporter type-2 (*SLC19A3*) and the human mitochondrial thiamine pyrophosphate transporter (*SLC25A19*). A schematic diagram of these proteins illustrating 25 and 3 mutations reported to date. †: novel unreported mutations identified in this study, ‡: most common mutation.

Figure 6. Clinical, biochemical and radiological characteristics of patients with inherited thiamine defects (N=79) compared to secondary thiamine deficiency (N=153)

Tables

Table 1. Suggested criteria for diagnosis of inherited thiamine defects with prominent neurologic involvement

Supplementary material

Supplementary Table 1. Demographic, clinic, genetic and radiologic features of 78 patients with *SLC19A3*, *SLC25A19* and *TPK1* deficiency

*Other sibling affected not included in this study, NA = Not available, **SYMPTOMS**

AT ONSET/ OUTCOME: Encephalopathy (E), Paroxysmal Ataxia (A), Hypotonia (H), Tremor (T), Dystonia (D), Status dystonicus (Sd), Chorea (Ch), Opisthotonus (O), Rigidity (Akinetic-rigid syndrome) (Ak), Spasticity (S), Nystagmus (N), Strabismus (Sb), Ptosis (Pt), Ophthalmoplegia (Oph), Diplopia (D), Vertigo (V), Hypoaesthesia (Hy) Dysarthria (Dth), Dysphagia (Dph), Weight loss (W), Respiratory failure (Res), Liver disease (Hep), Rhabdomyolysis (Rab), Dysautonomia (Dys), Jaundice (J), Seizure – including focal, generalized and infantile spasms (Sz), Peripheral neuropathy (Pn), Developmental arrest (Rg), Movement disorder (MD), Spasticity (S), Intellectual disability (ID), Scoliosis (Sch), microcephaly (m). **ABNORMAL REGIONS ON NEUROIMAGING:** Caudate (C), Putamen (P), Globus pallidus (Gp), Thalamus (T), Corticosubcortical (Cs), Cerebellum (Cb), Brainstem (B), Periqueductal (Pq), Spinal cord (SC), Lactate on MRS (Lactate), Cerebral atrophy (CA), Cerebellar atrophy (CbA). **PHENOTYPES** Leigh Syndrome (LS), Biotin responsive basal ganglia disease (BTRBGD), Asymptomatic

CASE	REF	CONSAUINITY- FAMILY HISTORY (Yes (Y), No (N))	ETHNICITY	ONSET/ CURRENT AGE (Years)	MUTATION ALLELE 1	MUTATION ALLELE 2	PHENOTYPES	SYMPTOMS at onset	TRIGGER EVENT at onset	ABNORMAL REGIONS ON NEUROIMAGING	OUTCOME	DISABILI TY SCORE
P1	27	N	White European-Spanish	13mo/-	c.1079dupT/p.L360fs*11	c.980-14A>G/p.G327Dfs*8	LS	E, H, T, D, Ch, O, N, J, Hep, W, Res, Dph, A	Viral infection	C,P,T,Cs, Lactate	Deceased at 14 mo	-
P2	27	Y	Arab-Moroccan	1mo/3y	c.68 G>T/p.G23V	c.68 G>T/p.G23V	LS	E, H, T, D, O, S, Dph	None	P, T, Cs, Lactate	Alive-MD, E, S, M	16
P3	27	N-sibP4	White European-Spanish	17y/25y	c.74dupT/p.S26Lfs*19	c.980-14 A>G/p.G327Dfs*8	BTRBGD	E, H, T, D, Sd, Ak, S, N, Rab, Dys, Dth, Hy, V, D, Pt	Profuse exercise	C,P,T,Cs	Alive-normal, D	5
P4	27	N-sibP3	White European-Spanish	4y/4y	c.74dupT/p.S26Lfs*19	c.980-14 A>G/p.G327Dfs*8	BTRBGD	E, H, T, D, Dph, Dth	None	C,P,T, Lactate	Alive-normal	1
P5	7	Y-sibP6	Arab-Saudi Arabian	2y/7y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S	Viral infection	C,P,T, Cs, Lactate	Alive-normal	0
P6	7	Y-sibP5	Arab-Saudi Arabian	8y/12y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S, Oph, Dth, Dph, Sz	Viral infection	C,P,T, Cs, Cb, B, Pq, SC, Lactate	Alive-normal	3
P7	7	Y-sibP14	Arab-Saudi Arabian	13y/-	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, T, D, S, Oph, Dth, Dph, Res, Rab, Sz	Viral infection	C,P,T, Cs, Cb, B, Pq, SC, Lactate	Deceased at 13 y	-
P8	7	Y	Arab-Saudi Arabian	13y/16	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, T, D, S Oph, Dth, Dph, Res, Sz	Viral infection	C,P,T, Cs, Cb, B, Pq, Lactate	Alive-S, ID, D	29
P9	7	Y-sibP10	Arab-Saudi Arabian	6y/10y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S, Dth, Dph	Viral infection	C,P, Lactate	Alive-ID, D	10
P10	7	Y-sibP9	Arab-Saudi Arabian	-/16y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	NA	NA	C,P,T, Cs, Cb, B, Pq, SC, Lactate	Alive-not known	-
P11	7	Y	Arab-Saudi Arabian	50mo/8y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S, Oph, Dth, Dph, Sz	None	C,P,T, Cs, Cb, B, Pq, SC, Lactate	Alive-normal	0
P12	7	Y	Arab-Saudi Arabian	-/7y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	NA	NA	C, P, T, B, SC	Alive-normal	-
P13	7	Y	Arab-Saudi Arabian	8y/12y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Oph, Dth, Dph, Sz	Viral infection	C,P,T, Cs, Cb, B, SC, Lactate	Alive-normal	0
P14	7	Y-sibP7	Arab-Saudi Arabian	11y/32y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, D, S, Dth	None	C, P	Alive-S, ID, D	10
P15	7	Y	Arab-Saudi Arabian	11y/13y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Oph, Dth, Dph, Sz	Viral infection	C,P,T, Cs, Cb, SC	Alive-normal, D	6
P16	7	Y	Arab-Saudi Arabian	2y/5y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Viral infection	C,P,T, Cs, Cb, B, SC, Lactate	Alive-S, ID, D	8
P17	7	Y	Arab-Saudi Arabian	3.5y/15y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Viral infection	C, P	Alive-ID, D	8
P18	7	Y	Arab-Saudi Arabian	11y/13y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S, Oph, Dth	Viral infection	C,P,T, Cb	Alive-normal	2
P19	7	Y,*	Arab-Saudi Arabian	3y/6y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Trauma	C,P,T, Cs	Alive-normal	2
P20	7	Y-P21	Arab-Saudi Arabian	8y/11y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Oph, Dth, Dph, Sz	Viral infection	C, P, T, Cs, Cb, Pq, Lactate	Alive-normal	2
P21	7	Y-P20	Arab-Saudi Arabian	9y/13y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Sz	Trauma	C, P, T, Cs, Cb, Pq, Lactate	Alive-normal	2
P22	7	Y	Arab-Saudi Arabian	1y/4y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Viral infection	C, P	Alive-H, ID, D	14
P23	7	Y-P24	Arab-Saudi Arabian	5.5y/6y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, T, D, S, Dth, Dph, Sz	Viral infection	C, P, T, Cs, Cb, Pq, SC	Alive-S, ID	21
P24	7	Y-P23	Arab-Saudi Arabian	47mo/4,08y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph	Viral infection	C, P, T, Cs	Alive-normal	0
P25	7	Y	Arab-Saudi Arabian	5y/10,08y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Viral infection	C, P, T, Cs, Cb, Lactate	Alive-A	2
P26	7	Y	Arab-Saudi Arabian	1y/5y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Dth, Dph, Sz	Viral infection	C, P, T, Cs, Cb, B, Pq, SC, Lactate	Alive-m, H, ID, D	28
P27	7	Y	Arab-Saudi Arabian	15y/20y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, H, D, S, Oph, Dth	Trauma	C, P, T	Alive-normal, D	7
P28	22	N-P29	White European-Portuguese	29y/25y	c.74dupT/p.S26Lfs*19	c.980-14 A>G/p.G327Dfs*8	BTRBGD	E, D, N, Dth, Dph, Sz	Urinary tract infection	C, P, GP, Cs	Alive-D, S, Dth	5
P29	22	N-P28	White European-Portuguese	34y/40y	c.74dupT/p.S26Lfs*19	c.980-14 A>G/p.G327Dfs*8	BTRBGD	E, D, O, S, Oph, Dth, Dph, W	Viral infection	C, P, T, Cs, B	Alive-D, S, Dth, ID	23
P30	28	N-P31	White European-German	3y/-	c.280 T>C/p.W94R	c.1173-3992_1314+41del4175/p.Q393*fs	BTRBGD	E, A, Sd, O, S, Dth, Dph, Hep, Sz, D	Viral infection	C, P, T	Deceased at 12y	-
P31	28	N-P30	White European-German	9y/-	c.280 T>C/p.W94R	c.1173-3992_1314+41del4175/p.Q393*fs	BTRBGD	E, A, Sd, Ch, S, Dth, Dph, Res, Sz	Viral infection	C, P, T	Deceased at 13y	-
P32	NR	Y-P32	Arab-Moroccan	3.5y/9y	c.1264G>A/p.T422A	c.1264G>A/p.T422A	BTRBGD	E, H, T, Dth, Sz	None	C, P, T, Cs, Lactate	Alive-S, ID, D	10
P33	NR	Y-P33	Arab-Moroccan	1.5y/13y	c.1264G>A/p.T422A	c.1264G>A/p.T422A	BTRBGD	E, H, T, Sz	Gastroenteritis	C, P, Cs	Deceased at 13y	-
P34	29	N-P35, P36	Arab-Iraqi	19mo/4y	c.157A>G/p.N53D	c.157A>G/p.N53D	LS	E, A, H, D, Dth, Sb,	Pneumonia	C, P, T, Cs, Cb, Pq, Lactate	Alive-normal	0
P35	NR	N-P34, P36	Arab-Iraqi	Asymptomatic/ 0,16y	c.157A>G/p.N53D	c.157A>G/p.N53D	Asymptomatic	Asymptomatic	NA	NA	Alive-normal	-
P36	NR	N-P34, P35	Arab-Iraqi	Asymptomatic/ 0,16y	c.157A>G/p.N53D	c.157A>G/p.N53D	Asymptomatic	Asymptomatic	NA	NA	Alive-normal	-
P37	29	Y-P37	Arab-Kurdish	2y7mo/7y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	LS	E, H, T, S, Dph, Dth, A	Viral infection	C, P, T, Lactate	Alive-ID	-
P38	29	Y-P38	Arab-Kurdish	3mo/14y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, D, Dph, W,	None	C, T, Cs	Alive-ID, D	28
P39	2	Y-P40, P41	Arab-Moroccan	1mo/0,08y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 1mo	-
P40	2	Y-P39, P41	Arab-Moroccan	1mo/0,08y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 1mo	-
P41	2	Y-P39, P40	Arab-Moroccan	1mo/0,11y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	E, H, T, D, O, Ak, S, N	None	NA	Deceased at 6w	-

P42	2	Y-P43	Arab-Moroccan	0,08y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 1mo	-
P43	2	Y-P42	Arab-Moroccan	0,08y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 1mo	-
P44	2	N.*	Arab-Moroccan	1mo/20y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 20y	-
P45	2	N.*	Arab-Moroccan	1mo/15y	c.20C>A/p.S7*	c.20C>A/p.S7*	LS	NA	NA	NA	Deceased at 15y	-
P46	NR	N-P47	African/Afro-Caribbean	1mo/2mo	c.91T>C/p.S31P	c.516Het_delC/p.D173Tfs*35	LS	E, H, D, O, S	None	P, GP, T, Cs, B	Deceased at 2mo	-
P47	NR	N-P46	African/Afro-Caribbean	1mo/0,75y	c.91T>C/p.S31P	c.516Het_delC/p.D173Tfs*35	LS	E, H, T, D, O, S	None	P, GP, T, Cs, Ch, B	Deceased at 9mo	-
P48	NR	N	White European-Finnish	1mo/2y	c.541T>C/p.S181P	c.541T>C/p.S181P	LS	E, H, T, D, Sd, Ch, O, S	Viral infection	P, T, Cs, Cs, Lactate	Alive-H, m, D, ID	23
P49	NR	N	White European-Finnish	3,5mo/3,5y	c.541T>C/p.S181P	c.833T>C/p.L278P	LS	E, H, T, D, Sd, O	None	C, P, GP, T, Cs, Ch, B	Deceased at 3.5y	-
P50	26	Y.*	Arab-Saudi Arabian	3y/7y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, Dth,	Viral infection	C, P, Cs	Alive-normal	-
P51	26	Y	Arab-Saudi Arabian	3.5y/5y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	A	Viral infection	C, P, Cs	Alive-normal	-
P52	26	Y	Arab-Saudi Arabian	3mo/3y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, Sz	None	C, P, T, Cs, Ch, B	Alive-H, ID, Sz	-
P53	26	Y.*	Arab-Saudi Arabian	14mo/17y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	D, S, Dth, Sz	None	C, P	Alive-m, S, Sz, ID, D	9
P54	26	Y.*	Arab-Saudi Arabian	5mo/14y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, D, S, Dth, Sz	None	C, P, Cs, Ch	Alive-m, S, Sz, ID, D	26
P55	26	N	Arab-Saudi Arabian	1y/7y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, D, S, Sb, Sz, Dth	None	C, P, T, Cs	Alive-status not known	-
P56	26	Y	Arab-Saudi Arabian	5mo/4y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, Ch	Vaccination	C, P, Ch	Alive-A, ID, H	-
P57	26	Y	Arab-Saudi Arabian	3y/13y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, A, Dth, Sz	None	C, P, Cs, Pq	Alive-S, Sch, D	-
P58	26	N.*	Arab-Saudi Arabian	2y/24y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, S	Viral infection	C, P	Alive-D	-
P59	26	Y.*	Arab-Saudi Arabian	4y/25y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, A, Dth, Sz	None	C, P	Alive-D, Sz	-
P60	26	Y.*	Arab-Saudi Arabian	6y/13y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	S, Sz	None	C, P, Lactate	Alive-normal	-
P61	26	Y.*	Arab-Saudi Arabian	21mo/12y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	D, S, Dth, W, Sz	None	C, P, GP, Lactate	Alive-S, Sz, H, ID, D	29
P62	26	Y.*	Arab-Saudi Arabian	3y/4y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	A	Viral infection	C, P	Alive-normal	-
P63	26	Y.*	Arab-Saudi Arabian	3.5y/8y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	A, Dth, MD	None	C, Cs	Alive-normal	-
P64	26	Y	Arab-Saudi Arabian	4mo/18mo	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, S, Dph, Hep, Sz	Meningitis	C, P, Cs, Ch	Alive-Sz, ID, D	-
P65	26	Y	Arab-Saudi Arabian	23mo/4y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	Rg, S, Dth, Dph	Viral infection	C, P, CA, CbA	Alive-A, S, ID	-
P66	26	Y.*	Arab-Saudi Arabian	3y/5y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	Dth	None	NA	Alive-normal	-
P67	26	Y	Arab-Saudi Arabian	3y/3y7mo	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	Rg	None	NA	Alive-normal	-
P68	30	Y.*	Arab-Moroccan	3.5y/10y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, H, D, Sd, Ch, Sz, Dth	Viral infection	C, P, Cs, Lactate	Alive-ID	0
P69	30	Y	Arab-Kuwaiti	9.5y/14y	c.1264A>G/p.T422A	c.1264A>G/p.T422A	BTRBGD	E, Oph, D, Sd, Dth	Viral infection	C, P, T, Cs, Ch, SC	Alive-normal	0
P70	NR	N	White-Hispanic	23mo/27mo	c.74dupT/p.S26Lfs*19	c.503_505delCGT/p.Y169*	BTRBGD	E, T, Pt, V, Dph, Dth, Dys, S	Viral infection	C, P, Gp, T, Pq, Lactate	Alive-normal	-
P71	5	Y-P72, P73, P74	Arab-Israeli	6y/14y	c.373G>A/p.G125S	c.373G>A/p.G125S	Bilateral striatal necrosis and neuropathy	E, H, Dph, Dth	Febrile illness	C, P,	Alive-Pn, ADHD,	1
P72	5	Y-P71, P73, P74	Arab-Israeli	6.5y/14y	c.373G>A/p.G125S	c.373G>A/p.G125S	Bilateral striatal necrosis and neuropathy	E, H,	Febrile illness	C, P,	Alive-Pn, ADHD,	1
P73	5	Y-P71, P72, P74	Arab-Israeli	3.5y/26y	c.373G>A/p.G125S	c.373G>A/p.G125S	Bilateral striatal necrosis and neuropathy	E, H, Sd, Dph, Dth	Febrile illness	C, P,	Alive-Pn, MD	6
P74	5	Y-P71, P72, P73	Arab-Israeli	4y/24y	c.373G>A/p.G125S	c.373G>A/p.G125S	Bilateral striatal necrosis and neuropathy	E, H, Sd, Dph, Dth	Febrile illness	C, P, T	Alive-Pn, MD	3
P75	NR	Y	White European-German	20mo/21y	c.580T>C/p.S194P	c.580T>C/p.S194P	Bilateral striatal necrosis	E, T, D, O, V, A, Dph	Otitis media	C, P, Gp, Cs	Alive -normal	0
P76	9	Y-P77	Asian-Chinese	6mo/-	c604T>G/p.W202G	c604T>G/p.W202G	LS	E,H,S, Hy, Dph, Res, Sz	Viral infection	C, P, GP, T, B, Pq, SC, Cb-dentate	Deceased at 29 mo	-
P77	9	Y-P76	Asian-Chinese	4mo/4y5mo	c604T>G/p.W202G	c604T>G/p.W202G	LS	E, H, D, S, Sb, Hy, Dph	Febrile illness	P, T, Cb-dentate	Alive – S, H, ID	23
P78	NR	N.*	White European-Finnish	1mo/-	c.365T>C/p.I122T	c.365T>C/p.I122T	LS	H, Hep, Sz	N	P, Gp, Cb-dentate	Deceased at 6 mo	-
P79	10	Y	Indian	2,5y/7y	c.479C>T/p.S160L	c.479C>T/p.S160L	LS	S	Viral infection	Cb-dentate	Alive – S, MD, M, ID	14

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AT ONSET/ OUTCOME: Encephalopathy (E), Paroxysmal Ataxia (A), Hypotonia (H), Tremor (T), Dystonia (D), Status dystonicus (Sd), Chorea (Ch), Opisthotonus (O), Rigidity (Akinetic-rigid syndrome) (Ak), Spasticity (S), Nystagmus (N), Strabismus (Sb), Ptosis (Pt), Ophthalmoplegia (Oph), Diplopia (D), Vertigo (V), Hypoaesthesia (Hy) Dysarthria (Dth), Dysphagia (Dph), Weight loss (W), Respiratory failure (Res), Liver disease (Hep), Rhabdomyolysis (Rab), Dysautonomia (Dys), Jaundice (J), Seizure – including focal, generalized and infantile spasms (Sz), Peripheral neuropathy (Pn), Developmental arrest (Rg), Movement disorder (MD), Spasticity (S), Intellectual disability (ID), Scoliosis (Sch), microcephaly (m). **ABNORMAL REGIONS ON NEUROIMAGING:** Caudate (C), Putamen (P), Globus pallidus (Gp), Thalamus (T), Corticosubcortical (Cs), Cerebellum (Cb), Brainstem (B), Periqueductal (Pq), Spinal cord (SC), Lactate on MRS (Lactate), Cerebral atrophy (CA), Cerebellar atrophy (CbA). **PHENOTYPES** Leigh Syndrome (LS), Biotin responsive basal ganglia disease (BTRBGD), Asymptomatic

Reference of patients with PDHc deficiency

To identify previously published PDHc deficiency cases a systematic literature search was conducted in PubMed in January 2017 using the search terms “Pyruvate dehydrogenase complex deficiency”, “pyruvate dehydrogenase complex”, “PDHA1”, “PDHB” and “PDHX”. The search was limited to studies in humans published after 1.1.1990 (considering availability in our electronic library). We restricted the search to articles in English. 75 full-text records were retrieved, describing 324 cases with PDHc deficiency. The cases were used to ascertain genotypes, sex and for Kaplan- Meier survival analysis.

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Figure 1. Thiamine metabolism
243x182mm (300 x 300 DPI)

Accept

Figure 2. Clinical feature and MRI

243x182mm (300 x 300 DPI)

Figure 3. MRI patterns in patients with secondary and inherited thiamine defects.

170x126mm (300 x 300 DPI)

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Figure 4. Kaplan cruves and timeframe

243x182mm (300 x 300 DPI)

hTHTR2: *SLC19A3*

hMTPPT: *SLC25A19*

Figure 5. Pathogenic mutations
243x182mm (300 x 300 DPI)

Accept

	A. Primary causes (SLC19A3 N=70; SLC25A19 N=5; TPK1 N=4)	B. Secondary causes (beriberi N=88; Wernicke encephalopathy N=65)
Age at onset	SLC19A3: 1 month – 34 years, SLC25A19: 20 months – 6.5 years and TPK1: 1 month – 2.5 years	Beriberi (two weeks- 1 year), Wernicke's encephalopathy (19 months – 17 years)
Consanguinity	SLC19A3 and TPK1: 73 -75%, SLC25A19: 100%	Non-consanguinity reported
Triggers or predisposing factors	Trigger events in 55% of patients: viral or bacterial infections, trauma, profuse exercise and vaccination	Predisposing factor in 100% patients. Beriberi: breastfeeding by mothers with inadequate intake of thiamine or feeding with low thiamine-content formula. Wernicke's encephalopathy: gastrointestinal surgical procedures, prolonged total parenteral nutrition without thiamine, malignancies, organ transplantation, pancreatitis, restricted diet (soy milk, autism spectrum disorder)
Clinical pictures	Recurrent encephalopathy (Leigh syndrome phenotype) is common in all three genetics defects. No cardiovascular or respiratory symptoms are observed. Other symptoms: SLC19A3: dystonia, seizure, spasticity, dysphagia, dysarthria, ophthalmoplegia, nystagmus, strabismus, akinetic-rigid syndrome, psychomotor regression and ataxia, SLC25A19: flaccid tetraparesis and peripheral neuropathy and TPK1: ataxia, dystonia and developmental delay	Beriberi symptoms: systemic (fever, vomiting, diarrhea and anorexia), neurologic (altered sensorium, persistent hoarse cry, ptosis, nystagmus, seizure, hypotonia, divergent squint, peripheral and central neuropathy), cardiovascular (shock, tachycardia and edema) and respiratory symptoms (acidotic breathing and respiratory distress syndrome) Wernicke's encephalopathy triad: acute or subacute ataxia, altered consciousness, and ophthalmoparesis
Biochemical abnormalities	SLC19A3: metabolic acidosis (16%), increased lactate in blood (50%) and CSF (17%) and normal thiamine blood levels, and increased CSF lactate SLC25A19 100% and TPK1 0%	84% (11/13) series report metabolic acidosis, increased lactate in blood, urine and/or CSF, 100% (16/16) detect low thiamine blood levels.
MRI abnormalities (in order of frequency)	SLC19A3: putamen, caudate, cerebral cortex, ventromedial thalamus, cerebellum, brainstem, periaqueductal region, spinal cord and globus pallidus, SLC25A19: putamen and caudate and TPK1: basal ganglia, cerebellum and dentate nuclei.	Common (>50% series): dorsomedial thalamus, periaqueductal region, mammillary bodies, caudate, putamen, and tectal plate. Uncommon (<50%): cranial nerve nuclei, cerebral cortex, and hypothalamus and cerebellum.
Treatment and response to treatment	SLC19A3 (Td: 5-55 mg/kg/d): 86% presented clinical recovery in the following hours or days, 3 patients did not improve at all, SLC25A19 (Td: 400 – 600 mg/day): avoid decompensation and TPK1 (Td: 15 mg/kg/d): variable response.	Initially Td 50 – 1500 mg/day (oral or intra-venous), then oral treatment for 2-3 weeks. Complete biochemical and clinical improvement in a few hours.
Sequels	SLC19A3: 38% mild to moderate intellectual disability, ataxia, seizure and dystonia, SLC25A19: polyneuropathy, and TPK1: 100% severe sequels	<6% mild to severe intellectual and motor disability, ataxia, seizures. Radiological improvement in 2 - 3 months in the majority of cases.
Mortality	SLC25A19 - BSDPP phenotype: 0%, SLC19A3: 21% and TPK1: 50%	5- 20%

Figure 6. Inherited thiamine defects

170x126mm (300 x 300 DPI)

Table 1. Suggested criteria for the diagnosis of inherited thiamine defects with prominent neurologic involvement**Required****1. Clinical criteria**

- a. **SLC19A3**: Acute or recurrent episodes of encephalopathy (decreased consciousness, irritability) with 2 or more of the following: 1) dystonia, 2) hypotonia, 3) bulbar dysfunction, 4) ataxia and 5) seizures. Of note 16% of patients may have an insidious onset of symptoms (psychomotor regression, clumsy or abnormal gait, stiff limbs).
- b. **SLC25A19**: Acute or recurrent episodes of encephalopathy with: 1) progressive peripheral neuropathy, or 2) severe congenital microcephaly with brain malformations.
- c. **TPK1**: Acute or recurrent episodes of encephalopathy, with 2 or more of the following: 1) dystonia, 2) hypotonia, 3) ataxia, 4) seizures and 5) developmental delay. Of note some patients may have a non-episodic early onset global developmental delay.

2. Biochemical criteria

- a. Normal total thiamine blood levels
- b. Low free-thiamine in CSF and/or fibroblasts (*SLC19A3*)
- c. Low TDP in blood, muscle and/or fibroblasts (*TPK1*)
- d. High excretion of alpha-ketoglutaric acid in urine (common in *TPK1* and *SLC25A19*, rare in *SLC19A3*).

3. Radiological criteria

- a. MRI pattern compatible with Leigh syndrome (*SLC19A3*, *SLC25A19*, *TPK1*) or Wernicke encephalopathy (*SLC19A3*)
 - i. **SLC19A3**: Symmetrical T2W hyperintensity of caudate, putamen, cortico-subcortical areas and/or ventromedial thalamus. No involvement of mammillary bodies.
 - ii. **SLC25A19**: Symmetrical T2W hyperintensity in the caudate and putamen.
 - iii. **TPK1**: Symmetrical T2W hyperintensity in basal ganglia and cerebellum (dentate nuclei).

4. Therapeutic criteria

- a. Clinical improvement after thiamine supplementation.

Supportive

1. Consanguinity
2. Trigger event (infection, vaccination, trauma, intense physical activity, etc.).
3. Absence of predisposing factors of beriberi or Wernicke encephalopathy.
4. Absence of systemic features of mitochondrial disease (cardiomyopathy, arrhythmia/conduction defects, renal tubulopathy or dysmorphic features).
5. Increased lactate in blood and/or CSF.
6. Normal OXPHOS and PDHc activity in muscle and fibroblast.
7. Increased lactate on MRS.

Figure 1. Thiamine metabolism (Black and White version)

243x182mm (300 x 300 DPI)

Accept

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