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Expanding the clinical and molecular spectrum of thiamine pyrophosphokinase deficiency: A treatable neurological disorder caused by *TPK1* mutations

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ABSTRACT

Thiamine pyrophosphokinase (TPK) produces thiamine pyrophosphate, a cofactor for a number of enzymes, including pyruvate dehydrogenase and 2-ketoglutarate dehydrogenase. Episodic encephalopathy type thiamine metabolism dysfunction (OMIM 614458) due to *TPK1* mutations is a recently described rare disorder. The mechanism of the disease, its phenotype and treatment are not entirely clear.

We present two patients with novel homozygous *TPK1* mutations (Patient 1 with p.Ser160Leu and Patient 2 with p.Asp222His). Unlike the previously described phenotype, Patient 2 presented with a Leigh syndrome like non-episodic early-onset global developmental delay, thus extending the phenotypic spectrum of the disorder. We, therefore, propose that TPK deficiency may be a better name for the condition. The two cases help to further refine the neuroradiological features of TPK deficiency and show that MRI changes can be either fleeting or progressive and can affect either white or gray matter. We also show that in some cases lactic acidosis can be absent and 2-ketoglutaric aciduria may be the only biochemical marker. Furthermore, we have established the assays for TPK enzyme activity measurement and thiamine pyrophosphate quantification in frozen muscle and blood. These tests will help to diagnose or confirm the diagnosis of TPK deficiency in a clinical setting.

Early thiamine supplementation prevented encephalopathic episodes and improved developmental progression of Patient 1, emphasizing the importance of early diagnosis and treatment of TPK deficiency. We present evidence suggesting that thiamine supplementation may rescue TPK enzyme activity.

Lastly, in silico protein structural analysis shows that the p.Ser160Leu mutation is predicted to interfere with TPK dimerization, which may be a novel mechanism for the disease.

1. Introduction

Thiamine pyrophosphokinase (TPK, EC 2.7.6.2.) transfers a pyrophosphate group from adenosine triphosphate (ATP) to thiamine to produce its active form, thiamine pyrophosphate (TPP) in the cytosol [1]. TPP is a cofactor for enzymes important in a range of fundamental

Abbreviations: ATP, adenosine triphosphate; MRI, magnetic resonance imaging; THDM5, episodic encephalopathy type thiamine metabolism dysfunction; TPK, thiamine pyrophosphokinase; TPP, thiamine pyrophosphate.

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processes such as cellular respiration (pyruvate dehydrogenase and 2-ketoglutarate dehydrogenase) and in providing substrates for synthesis of nucleic acids, nucleotides, fatty acids and steroids (transketolase in the pentose phosphate pathway). It is needed for the catabolism of amino acids (branched-chain α-keto acid dehydrogenase), phytanic acid and 2-hydroxy straight chain fatty acids (2-hydroxyphytanoyl-CoA lyase). A number of defects in thiamine transport and metabolism are now known [2] and *TPK1* mutations resulting in episodic encephalopathy type thiamine metabolism dysfunction (THDM5, OMIM 614458) is the most recently described disorder of this group [3]. We present two new patients with *TPK1* mutations providing novel clinical and biological insights into the condition.

2. Case histories

Patient 1 (P1) is a male child born to first cousin parents of Indian origin. He presented at 30 months of age during a viral illness with loss of ability to walk and ataxia. On examination he had brisk deep tendon reflexes. He recovered gradually. At 32 months he presented similarly with chicken pox, followed by development of extra-pyramidal features, upper motor neuron signs and fluctuating hypertonia during recovery. His vocabulary reduced to ten words and he became emotionally labile. At 36 months he developed encephalopathy and fluctuating awareness during an episode of gastroenteritis. During recovery his vocabulary reduced to three words and he became naso-gastric tube dependent.

Patient 2 (P2) is an eight-year-old daughter of unrelated German parents. She presented during infancy with feeding difficulties, delayed motor development, severe truncal hypotonia, hypertonia of the limbs and brisk reflexes.

Investigations performed in P1 and P2 are summarized in Tables S1 and S2 respectively. Their brain magnetic resonance imaging (MRI) images are shown in Fig. 1. 2-Ketoglutaric aciduria was observed in both patients. Lactate levels were elevated in P2 (with low lactate:pyruvate ratio) but were normal in P1. Muscle biopsy studies of P2 revealed decreased utilization of pyruvate but normal pyruvate dehydrogenase complex (PDHC) activity, suggesting a defect in pyruvate transport or a disorder of the cofactor metabolism.

3. Materials and methods

TPK1 Sanger sequencing, TPP quantification and TPK immunoblotting were performed as described previously [3].

3.1. Cloning of mutant TPK1

Human wild type TPK1 was expressed from the vector PRSET-hTPK1 in *Escherichia coli* as reported previously [4]. Mutant *TPK1* was amplified from patient cDNA and PCR amplification with the forward primer 5′-cgggatccgATGGAGCATGCCTTTACC-3′ that contains a BamHI site and the reverse primer 5′-gaagatctTTAGCTTTTG ATGGCCATGG-3′ that contains a BglII site. The mutant *TPK1* was cloned into this vector by replacing the wild type sequence. The final constructs were sequenced and the mutations, c.479C>T (p.Ser160Leu, Patient 1) and c.664G>C (p.Asp222His, Patient 2) were confirmed.

3.2. Expression and purification of TPK1 protein

The *E. coli* strain BL21(DE3)pLysS (Promega) was transformed with either mutant or wild type *TPK1* on pRSET B and grown on LB medium containing 50 μl/ml chloramphenicol and 100 μl/ml ampicillin. For expression of the recombinant protein, 200 ml of this medium containing 1 mmol/l isopropyl-β-D-thiogalactopyranoside (IPTG) was inoculated with an overnight preculture at an optical density at 600 nm of 0.1

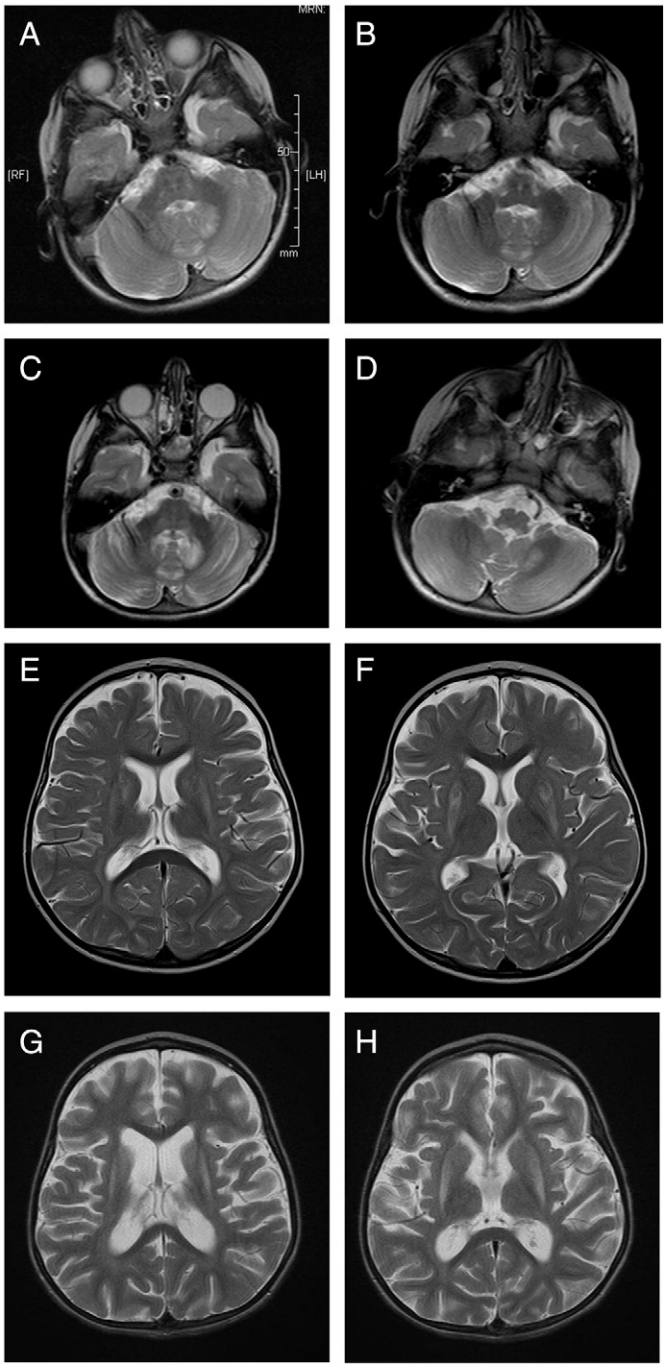

Fig. 1. Axial T2 brain magnetic resonance imaging. A) Hyper-intense signals in the dentate nucleus (P1, first episode, 30 months). B) Resolution of the earlier changes (P1, second episode, 32 months). C and D) Pyramidal tract signal changes in the medulla and left dentate nucleus (P1, third episode, 36 months). E and F) Delayed myelination and altered density in the basal ganglia (P2, 16 months). G and H) Enlarged extra-axial CSF spaces suggesting cortical atrophy (P2, 41 months).

and grown for 2 h. The cells were harvested by centrifugation, washed once and resuspended in 20 ml lysis buffer/wash buffer (50 mmol/l sodium phosphate, 300 mmol/l sodium chloride, 10 mmol/l imidazole, pH 7.4). Cells were disrupted by sonication and crude protein extracts were obtained by collecting the supernatant after centrifugation for 5 min at 10,000 *g*. Sonication and all following steps were performed under cooling with ice. Since the recombinant human TPK contains an N-terminal His-tag we purified the crude extracts with HisPur cobalt spin columns (1 ml columns, Pierce Biotechnology) according to the manufacturer's instructions. After washing with 50 resin volumes of