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**Supplemental Data**

# Thiamine Pyrophosphokinase Deficiency in Encephalopathic Children with Defects in the Pyruvate Oxidation Pathway

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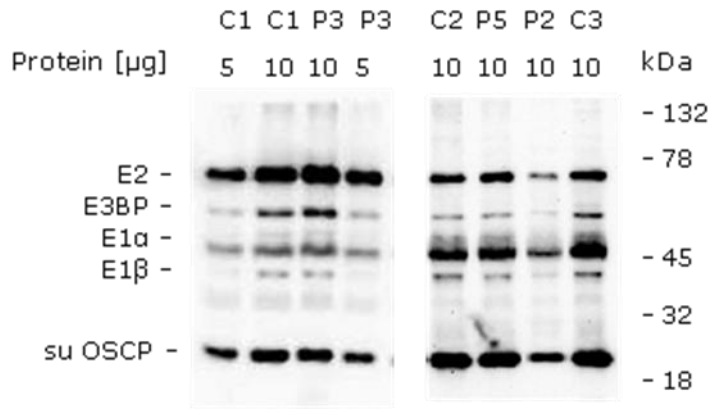


**Figure S1.** Western blot analysis with an antibody cocktail against subunits of the pyruvate dehydrogenase complex and subunit OSCP of the ATP synthase showed no abnormal staining of the affected individuals (P) compared to control (C) samples. Post nuclear supernatants from muscle were applied in the indicated amount of protein.


**Figure S2.** The amino acids affected by the missense mutations found in the affected individuals are shown as space filled molecules in the crystalline structure of the human thiamin pyrophosphokinase (protein data bank, http://www.rcsb.org, accession number 3S4Y). The homozygous mutation p.Leu40Pro was found in the affected individuals 3+4, affects an amino acid located between the first helix of the protein and a β-sheet (A). The compound heterozygous mutation p.Asn50His of affected individuals 1+2 affects a conserved amino acid in the second helix of the protein (B). The compound heterozygous mutation p.Asn219Ser affects a highly conserved amino acid located in a β-sheet, which is close to the TPP substrate (C). Illustrations were prepared with the Jmol open-source software (http://www.jmol.org/).

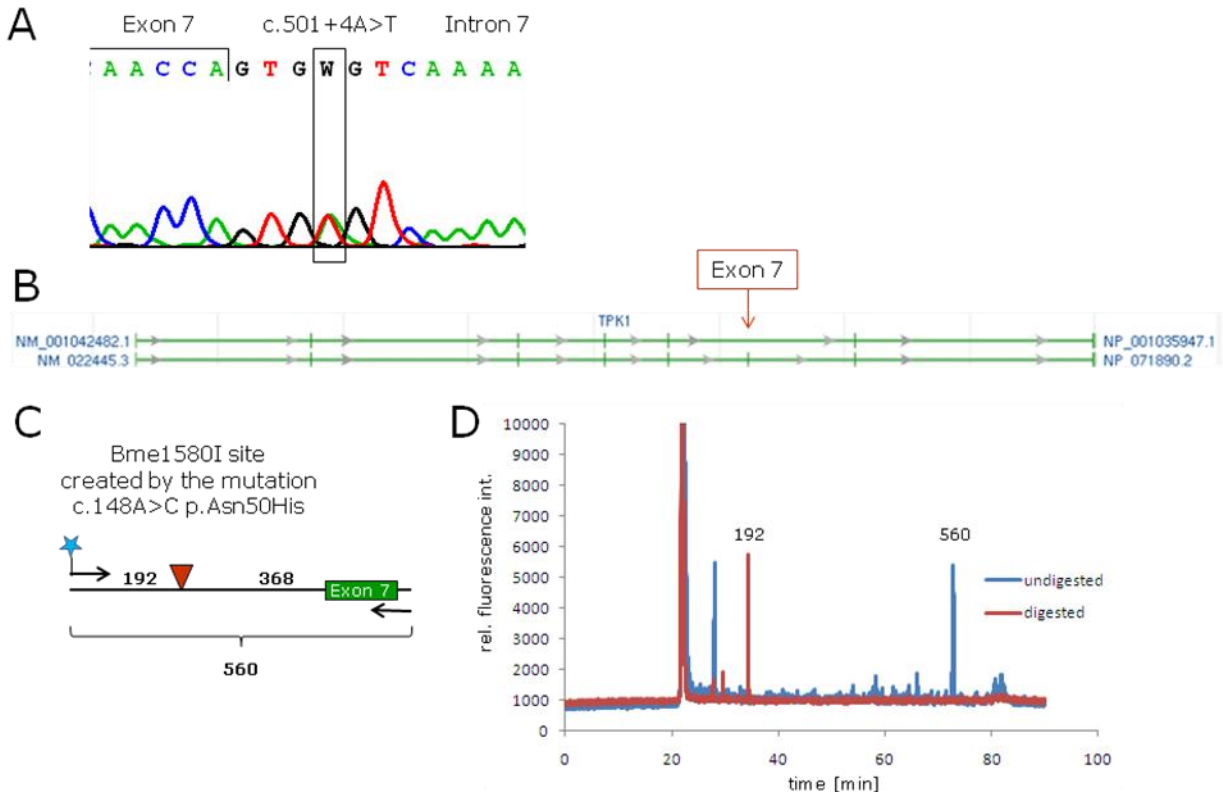


**Figure S3.** The mutation c.501+4A>T (A) affects the splice donor site of intron 7 found in the enzymatically active form of TPK1 RefSeq NP_071890.2 (B). This mutation disables the splicing of exon 7, a 560 bp cDNA product created by a Cy5-labelled forward primer from exon 1 and a reverse exon 7/exon 8 overlapping primer (C). This PCR product is fully digested to a 192 bp fragment by the restriction enzyme Bme1580I, which is specific for the missense mutation c.148A>C located on the other allele (D).