

Bi-allelic Truncating Mutations in *TANGO2* Cause Infancy-Onset Recurrent Metabolic Crises with Encephalocardiomyopathy

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Molecular diagnosis of mitochondrial disorders is challenging because of extreme clinical and genetic heterogeneity. By exome sequencing, we identified three different bi-allelic truncating mutations in *TANGO2* in three unrelated individuals with infancy-onset episodic metabolic crises characterized by encephalopathy, hypoglycemia, rhabdomyolysis, arrhythmias, and laboratory findings suggestive of a defect in mitochondrial fatty acid oxidation. Over the course of the disease, all individuals developed global brain atrophy with cognitive impairment and pyramidal signs. *TANGO2* (transport and Golgi organization 2) encodes a protein with a putative function in redistribution of Golgi membranes into the endoplasmic reticulum in *Drosophila* and a mitochondrial localization has been confirmed in mice. Investigation of palmitate-dependent respiration in mutant fibroblasts showed evidence of a functional defect in mitochondrial β -oxidation. Our results establish *TANGO2* deficiency as a clinically recognizable cause of pediatric disease with multi-organ involvement.

Hereditary forms of pediatric metabolic myopathies presenting with recurrent myoglobinuria and metabolic crises have been linked to genetic defects resulting in a shortage of muscle energy. Affected pathways include glycogen and (long-chain) fatty acid metabolism as well as mitochondrial ATP production.¹ In combination with a detailed clinical phenotype, exome sequencing is widely applied for diagnostic evaluation of individuals with suspected inborn errors of metabolism and allows the identification of novel disease-implicated genes.^{2,3} However, in a significant fraction of affected individuals, the genetic defect underpinning the clinical phenotype remains elusive. Explanations include the existence of pathogenic sequence variants in off-target genomic regions or sequence alterations such as copy-number variations (CNVs) or chromosomal rearrangements that are difficult to detect in exome datasets.

Here we report on a joint analysis of exome datasets of three unrelated individuals sharing distinct phenotypic features using an analytical pipeline able to detect clinically relevant CNVs. The pivotal features of the affected individuals' disease presentations were recurrent metabolic encephalomyopathic crises hallmarked by laboratory findings of hypoglycemia, elevated plasma CK ac-

tivity, lactic acidosis, and increased acylcarnitines as well as massive urinary excretion of lactate, ketones, and dicarboxylic acids. The severity and duration of such crises were variable and response to intravenous glucose has been observed. Prior to the first crisis, global developmental delay as well as cortical signs were observed. Although the clinical condition stabilized in the interval, the overall disease course was characterized by progressive neurodegeneration with epilepsy, cognitive impairment, pyramidal and cerebellar signs, and loss of expressive language. Optic atrophy and sensorineural hearing impairment were observed in single individuals. Cardiac involvement with severe arrhythmias (torsade de pointes, long QT syndrome) was a consistent and potentially life-threatening manifestation. Increased TSH concentrations indicating hypothyroidism have been documented in all three affected individuals. Clinical and genetic findings are summarized in Table 1, pedigrees are shown in Figure 1, neuroimaging findings are provided in Figure 2, and detailed case reports are provided in the Supplemental Note. Informed consent was obtained from all affected individuals or their guardians in case of minor study participants. The study was approved by the ethical committee of the Technische Universität München.

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Table 1. Genetic and Clinical Findings in Individuals with Bi-allelic TANGO2 Variants

TANGO2 Variants		Phenotypic Features										Laboratory Findings during Crises						
ID	Sex	cDNA (NM_152906.5); Protein (NP_690870.3)	AO	Current Dev. Age	Episodic Met./Enceph. Crises	Myopathy (Weakn./Rhabd.)	Cardiac Arrhyth. (Rhabd.)	TSH Elevation	Ophthalmologic Findings	Cognitive Impairment	Epilepsy Spasticity	Brain Atrophy	Hypo-glycemia	Plasma Lactate Elevation	Hyper-CKemia	Ketonuria	Acyl-carnitines Elevated	Dicarboxylic Acids Elevated
F1:II.2 f		c.[4delT]; [(56+1_57-1)_(*1_?)del]; p.[Cys2Alafs*35];[?]	2.5 years	3.25 years	yes (2)	yes	yes	yes	normal	yes	yes	yes	yes	yes	yes	yes	yes	yes
F2:II.2 f		c.[418C>T]; [418C>T]; p.[Arg140*]; [Arg140*]	5 months	12.5 years	yes (4)	yes	yes	yes	optic atrophy	yes	yes	yes	yes	yes	yes	yes	yes	yes
F3:II.1 f		c.[(56+1_57-1)_(*1_?)del]; [(56+1_57-1)_(*1_?)del]; p.[?];[?]	3 years	25 years	ND	yes (> 5)	yes	yes	normal	yes (IQ 71)	yes	yes	mild	yes	yes	mild	yes	not reported

Abbreviations are as follows: f, female; AO, age of onset; ND, not determined; Dev., developmental; met., metabolic; enceph., encephalopathic; Weakn., weakness; Rhabd., rhabdomyolysis; arrhyth., arrhythmias; HyperCKemia; elevated CK activity in blood.

Whole-exome sequencing was performed on genomic DNA from three unrelated affected individuals and parents from family 3 as described previously.⁴ Using a frequency filter of minor allele frequency < 0.1% in our in-house database and public databases, we identified 10, 37, and 14 genes carrying potential compound heterozygous or homozygous variants in individuals F1:II.2, F2:II.2, and F3:II.1, respectively. In all individuals, *TANGO2* (GenBank: NM_152906.5) was the only gene carrying two predicted loss-of-function alleles (Figure 1). No additional individuals carrying potentially pathogenic bi-allelic *TANGO2* variants were detected in 6,000 in-house control exomes. Although the homozygous *TANGO2* stop variant c.418C>T (p.Arg140*) has been identified in individual F2:II.2 in a first-pass analysis, its clinical relevance initially remained unclear. The 34.6 kb exon 3–9 deletion present in individual F3:II.1 in a homozygous state (Figure S1) and in individual F1:II.2 in compound with a frameshift variant (c.4delT [p.Cys2Alafs*35]) was identified only during a second-pass analysis using a data analysis pipeline optimized for the detection of CNVs (ExomeDepth).⁵ All variants were confirmed in the affected individuals by Sanger sequencing or allele-specific PCR (for deletion exons 3–9) with parents being heterozygous carriers of one variant each, in line with an autosomal-recessive inheritance. None of the variants have been reported in a homozygous state in public databases (1000 Genomes, dbSNP 142) or an in-house database containing 6,000 exome datasets of individuals with unrelated phenotypes. The frameshift variant c.4delT was absent from in-house control exomes as well as ~120,000 alleles of the Exome Aggregation Consortium (ExAC) Server (Cambridge, MA [09/2015]). The stop gain variant c.418C>T has been detected only once in a heterozygous state in ExAC. The frequency of the 34.6 kb deletion observed in a homozygous as well as a compound heterozygous state could not be exactly determined in public databases due to insufficient annotation. However, in our in-house database containing 5,300 exome datasets being analyzed for CNVs, this deletion was found seven times only in a heterozygous state resulting in a MAF of 0.13%. Speculatively, this variant might therefore represent a more common disease allele in European populations. In summary, the identification of three different bi-allelic *TANGO2* loss-of-function variants in three unrelated individuals with a strikingly uniform clinical phenotype establishes *TANGO2* as a gene confidently implicated in human disease.

The physiologic function of *TANGO2* is not well understood. *TANGO2* was originally characterized in a genome-wide RNA-mediated interference screen in a *Drosophila* cell line.⁷ Depletion of *TANGO2* caused the Golgi apparatus to fuse with the endoplasmic reticulum (ER) and a putative role of *TANGO2* in redistributing Golgi membranes into the ER was postulated.⁶ Furthermore, a localization of *TANGO2* within the Golgi and cytosol has been suggested.⁶ We therefore investigated Golgi organization by immunostaining of Giantin (rabbit polyclonal

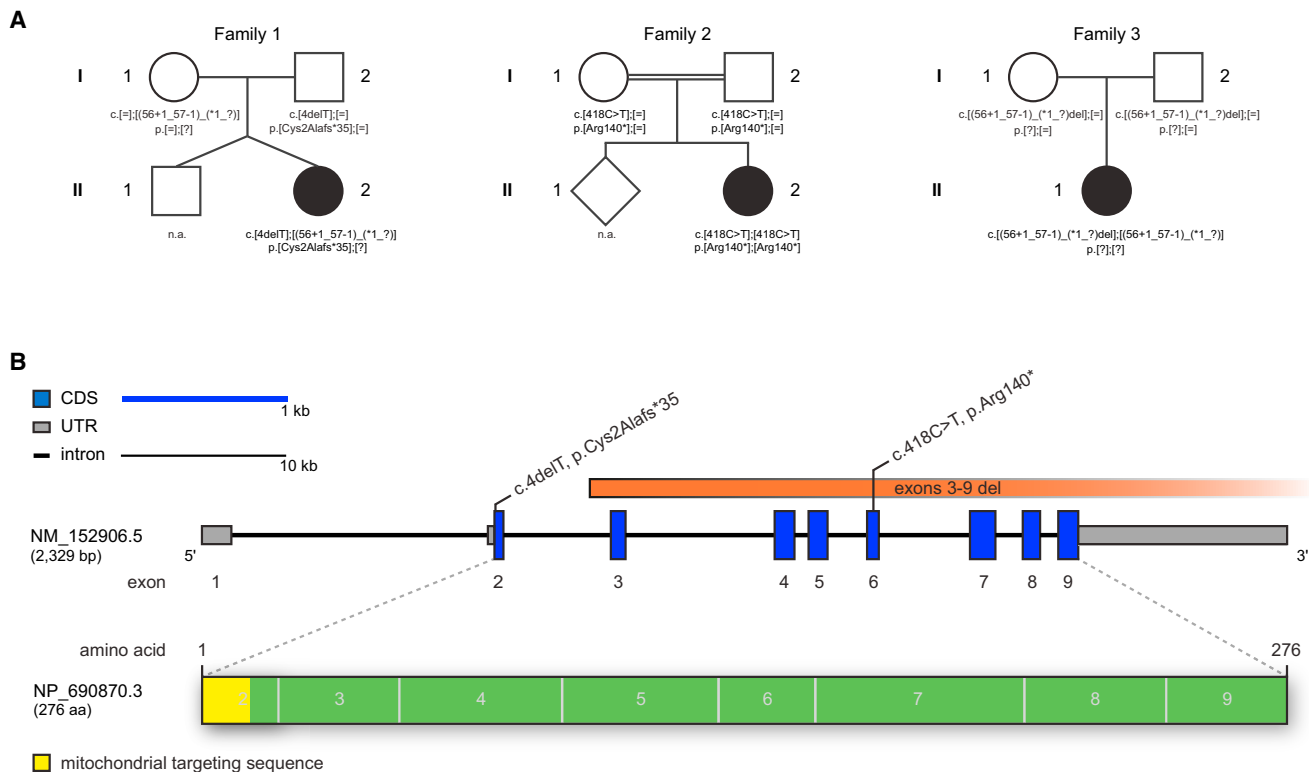


Figure 1. Pedigrees of Investigated Families and Structure of *TANGO2*

(A) Pedigrees of three families with mutations in *TANGO2*. Mutation status of affected (closed symbols) and healthy (open symbols) family members shown. n.a., not available.

(B) Gene structure of *TANGO2* with known protein domains of the gene product and localization of the identified mutations. Intronic regions are not drawn to scale.

anti-Giantin; 1:200; Abcam) and wheat germ agglutinin (WGA) fluorescence conjugate (W6748, ThermoFisher). We did not observe gross differences in Golgi organization and dimension measured by immunofluorescence between *TANGO2*-mutant and control fibroblasts (Figure S2). More recently, T10 (the mouse ortholog of *TANGO2*) has been studied in more detail in the context of 22q11.2 deletion syndrome, which is caused by heterozygous 1.5–3 Mb deletions on chromosome 22 at locus 11.2.⁷ With an estimated prevalence of 1 in 4,000, the 22q11.2 deletion syndrome is one of the most common microdeletion disorders and a major risk factor for schizophrenia. Interestingly, 9 of the 30 genes involved in 22q11.2 deletion syndrome (OMIM: 611867) have been shown to code for proteins localized to mitochondria (*COMT*, *UFD1L*, *DGCR8*, *MRPL40*, *PRODH*, *SLC25A1*, *TXNRD2*, *TANGO2*, and *ZDHHC8*).⁸ More specifically, Maynard et al. showed a co-localization of the T10-GFP fusion protein with a mitochondrially targeted mCherry protein but only minimal co-localization with Golgi and peroxisome.⁷ Furthermore, T10 was found robustly expressed in the 10 k mitochondria-enriched fractions of mice brain lysates.⁷ The protein sequences of T10 (GenBank: NP_613049.2) and *TANGO2* (GenBank: NP_690870.3) are 88% identical. The first 30 amino acids, which contain a predicted mitochondrial targeting

sequence (amino acids 1–13, MITOPROT), are 100% identical (Figure S3). Together, these observations suggest a mitochondrial localization of the gene products coded by *TANGO2*. The identification of the clinically relevant variant c.4delT, which affects only the coding sequence of the predicted mitochondrial isoforms, supports an involvement of mitochondrial metabolism in the pathogenesis of the disease.

Biochemical analyses of respiratory chain enzyme activities performed on muscle biopsies of individuals F2:II.2 and F3:II.1 showed normal results apart from a mild reduction in complex I activity in individual F2:II.2. Western blot studies in fibroblasts did not indicate a decrease in the amount of the mitochondrial respiratory chain complex I subunit NDUF8 (Figure S4). Furthermore, measurement of pyruvate-dependent oxygen consumption rates⁹ in fibroblast cell lines from individuals F1:II.2 and F2:II.2 was unremarkable. Together, these findings make it unlikely that primary dysfunction of the respiratory chain follows from *TANGO2* deficiency, although a transient impairment during metabolic crises cannot be excluded.

The metabolic signature observed in the investigated individuals is characterized by ketonuria, excretion of dicarboxylic acids (including glutaric and malonic acid) and lactate as well as a variable elevation of several acylcarnitine species. Just like the laboratory findings, the clinical

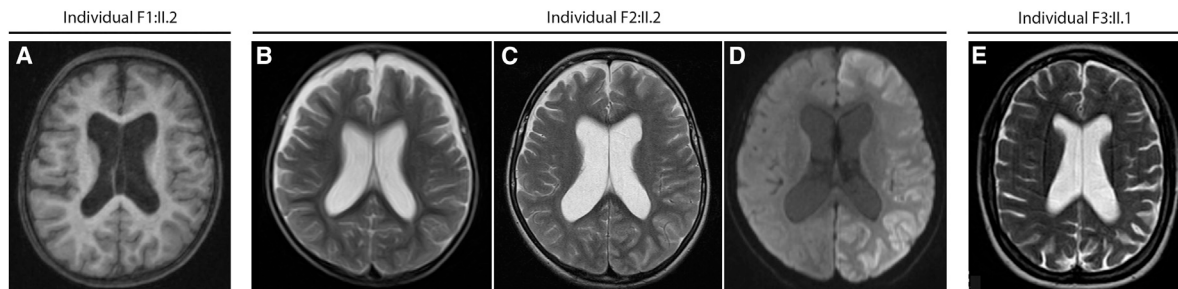


Figure 2. Neuroimaging Findings in *TANGO2* Mutant Individuals

(A–D) Brain MRIs of individuals F2:II.2 (A–C) and F3:II.1 (D). Although the individual MRI findings are nonspecific, the rather uniform pictures with widening of the ventricles should be noted when placing them side by side.

(A) Brain MRI (T₁-weighted image, axial view) at the age of 2 11/12 years, demonstrating mild widening of the ventricles suggestive of a generalized brain atrophy.

(B) Brain MRI (T₂-weighted image, axial view) at the age of 2 years, demonstrating mild widening of the ventricles and the bifrontal cerebrospinal fluid spaces.

(C) Brain MRI (T₂-weighted image, axial view) at the age of 6 years, showing no relevant changes compared to the earlier investigation.

(D) Diffusion-weighted imaging (axial view) at the age of 6 years during acute metabolic crisis and recurrent seizures showing extensive left-hemispheric cortical cytotoxic edema, possibly caused by prolonged focal status epilepticus.

(E) Brain MRI (T₂-weighted image, axial view) at the age of 22 years, demonstrating widening of the ventricles as a sign of generalized brain atrophy.

phenotype shows strong overlaps with disorders of mitochondrial fatty acid and ketone body metabolism such as carnitine palmitoyltransferase II (CPT2) deficiency (OMIM: 11600649, 608836, 255110) and multiple acyl-CoA dehydrogenase deficiency (MADD [OMIM: 11231680]).¹⁰ To test a potential impact of *TANGO2* deficiency on mitochondrial fatty acid oxidation, we tested palmitate-dependent mitochondrial respiration in control and mutant fibroblasts as described previously.¹¹ We observed a significant difference in palmitate-dependent oxygen consumption rate suggesting a functional defect in mitochondrial β -oxidation of fatty acids (Figure 3). Although further studies are needed to determine the physiological function of *TANGO2*, it is tempting to speculate

that *TANGO2* deficiency is associated with impaired electron transfer from FAD-dependent dehydrogenases to the mitochondrial respiratory chain or cofactor metabolism.

In conclusion, we describe bi-allelic loss-of-function variants in *TANGO2* as a cause of metabolic crises with onset in childhood potentially triggered by a catabolic metabolic state. It is possible that the individuals reported in this study present the severe end of the phenotypic spectrum. Our study suggests *TANGO2* deficiency as a differential diagnosis in cases of episodic encephalopathy and/or myopathy in infancy and childhood of unknown cause, especially in individuals with cardiac arrhythmias and laboratory findings suggestive of impaired mitochondrial fatty acid oxidation.

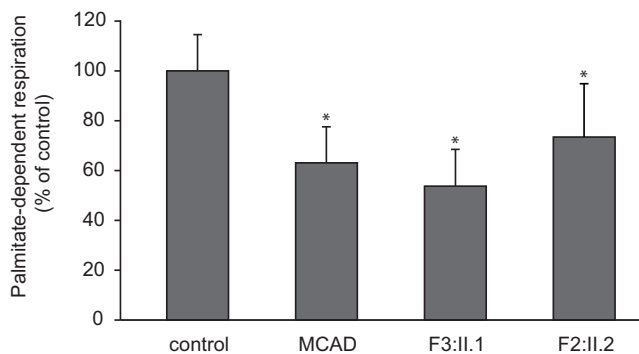


Figure 3. Investigation of Palmitate-Dependent Mitochondrial Respiration

Analysis of palmitate-dependent oxygen consumption rates (OCR) in fibroblast cell lines revealed impaired respiration in *TANGO2*-mutant cells in comparison to controls. A MCAD (medium-chain acyl-Coenzyme A dehydrogenase)-deficient cell line was used as a positive control. The experiment was performed several times with very similar results. The data are shown from one experiment performed with more than 12 replicates for each cell line grown and treated in parallel. Error bars indicate 1 SD from the mean. * $p < 0.001$; two-tailed unpaired t test.

Supplemental Data

Supplemental Data include case reports and three figures and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2015.12.009>.

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Web Resources

The URLs for the data presented herein are as follows:

ExAC Browser, <http://exac.broadinstitute.org/>

MitoProt, <http://ihg.gsf.de/ihg/mitoprot.html>

OMIM, <http://www.omim.org/>

RefSeq, <http://www.ncbi.nlm.nih.gov/RefSeq>

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