Table 1. Genetic and Clinical Findings in Individuals with *GTPBP3* Mutations

		GTPBP3 Mutations	OXPHOS Activities in Skeletal Muscle				Clinical Features				
ID	Sex	cDNA (NM_032620.3) and Protein (NP_116009.2)	RCC	% of Lower Control Range	Absolute Values	Reference Range	AO	Course	HCM	Histochemical COX Defect	Other Features
#49665[a,b]	male	c.[1291dupC; 1375G>A], p.[Pro430Argfs*86; Glu459Lys]	I	15%	0.025	0.17–0.56	10 years	alive 14 years	yes	ND	consanguineous parents (1st cousins), mild intellectual disability, fatigability, limited vision, lactic acidosis
			II	ND	ND	ND					
			II+III	normal	0.201	0.08–0.48					
			IV	24%	0.267	1.1–5.0					
#36349[b]	male	c.[1291dupC; 1375G>A], p.[Pro430Argfs*86; Glu459Lys]	I	no data	no data	no data	no data	alive 17 years	no data	no data	sibling of #49665 with similar clinical symptoms
			II								
			II+III								
			IV								
#66143[a]	male	c.[476A>T; 964G>C], p.[Glu159Val; Ala322Pro]	I	7%	0.01	0.19–0.48	2 years	alive 5 y ears	yes	ND	unrelated parents, sudden respiratory failure, lactic acidosis
			II	normal	0.10	0.07–0.12					
			II+III	normal	0.12	0.09–0.22					
			IV	28%	0.12	0.44–0.92					
#72425[a]	female	c.[484G>C; 673G>A; 964G>C], p.[Ala162Pro; Glu225Lys; Ala322Pro]	I	14%	0.015	0.11–0.30	3.5 months	died 8 months	DCM	yes	unrelated parents, cyanosis, hyporeactivity, DCM with residual ejection fraction of 20%, lactic acidosis
			II	normal	0.21	0.12–0.25					
			II+III	normal	0.06	0.006–0.14					
			IV	45%	0.76	1.7–4.0					
#75191[a]	female	c.[1009G>C; 1009G>C], p.[Asp337His; Asp337His]	I	31%	0.03	0.10–0.25	birth	died 1 day	yes	yes	unrelated parents, Kussmaul breathing, stridor, hypotonic, hyporeactivity, RVH, lactid acidosis
			II	normal	0.16	0.14–0.25					
			II+III	normal	0.12	0.13–0.25					
			IV	15%	0.09	0.60–1.48					
#76671	male	c.[665–2delA; 665–2delA], p.[Ala222Gly; Asp223_Ser270del; Ala222Gly; Asp223_Ser270del]	I	45%	0.05	0.11–0.30	birth	died 10 months	yes	yes	unrelated parents, hypotonia from birth, RVH, WPW, lactic acidosis
			II	normal	0.16	0.12–0.25					
			II+III	ND	ND	0.06–0.14					
			IV	17%	0.29	1.7–4.0					
#81471[a]	male	c.[424G>A; 424G>A], p.[Glu142Lys; Glu142Lys]	I	12%	0.012	0.104 ± 0.036	4 weeks	died 5 weeks	yes	yes	consanguineous parents, two healthy siblings, one miscarriage, FTT, poor weight gain and feeding, concentric LVH, lactic acidosis
			II	normal	0.098	0.145 ± 0.047					
			II+III	normal	0.850	0.544 ± 0.345					
			IV	17%	0.127	1.124 ± 0.511					

(*Continued on next page*)

Table 1. *Continued*

ID	Sex	*GTPBP3* Mutations: cDNA (NM_032620.3) and Protein (NP_116009.2)	OXPHOS Activities in Skeletal Muscle: RCC	% of Lower Control Range	Absolute Values	Reference Range	Clinical Features: AO	Course	HCM	Histochemical COX Defect	Other Features
#75168[a]	female	c.[770C>A; 770C>A], p.[Pro257His; Pro257His]	I	normal	no data	no data	2 years	alive 5 years	no	ND	consanguineous parents (1st cousins), developmental delay, epileptic seizures, intellectual disability, MRI hyperintense lesions of basal ganglia typical to Leigh syndrome, lactic acidosis
			II	normal							
			II+III	normal							
			IV	normal							
#82790[a]	female	c.[8G>T; 934_957del], p.[Arg3Leu; Gly312_Val319del]	I	36%	0.107	0.301 ± 0.05	1 year	alive 2 years	no	ND	unrelated parents, seizures, severe hypotonia, developmental delay, lactic acidosis
			II	normal	0.424	0.272 ± 0.05					
			II+III	normal	0.21	0.25 ± 0.093					
			IV	21%	0.008	0.035 ± 0.011					
#83904[a,c]	female	c.[32_33delinsGTG; 32_33delinsGTG], p.[Gln11Argfs*98; Gln11Argfs*98]	I	64%	4.2	6.5–17	1 week	died 9 months	yes	ND	consanguineous parents (1st cousins), lactic acidosis, WPW
			II	normal	16.1	13.6–45.7					
			II+III	normal	5.8	4.3–13.2					
			IV	25%	9.9	74–294					
#83905[a,c]	female	c.[32_33delinsGTG; 32_33delinsGTG], p.[Gln11Argfs*98; Gln11Argfs*98]	I	no data	no data	no data	birth	died 10 days	yes	ND	consanguineous parents (1st cousins), lactic acidosis, WPW
			II								
			II+III								
			IV								
#66654[a]	female	c.[673G>A; 964G>A]; [=] p.[Glu255Lys; Ala322Pro]; [=]	I	64%	0.09	0.14–0.35	1.5 months	alive	no	ND	intrauterine growth retardation, lactic acidosis, leukodystrophy, generalized hypotonia
			II	normal	0.19	0.18–0.41					
			II+III	90%	0.27	0.30–0.67					
			IV	normal	1.42	0.42–1.26					

Abbreviations are as follows: AO, age of onset; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; FTT, failure to thrive; LVH/RVH, left/right ventricular hypertrophy; ND, not determined; WPW, Wolff-Parkinson-White syndrome.
Mitochondrial respiratory chain complexes (RCC) in muscle: I, NADH:CoQ-oxidoreductase; II, succinate:CoQ-oxidoreductase; II+III, succinate:cytochrome c reductase; IV, cytochrome c oxidase (COX).
Enzyme activities were determined in muscle biopsies and normalized to citrate synthase (CS). Absolute values and reference ranges are given in [mU / mU CS].
[a]Investigated by exome sequencing.
[b,c]These individuals are siblings.

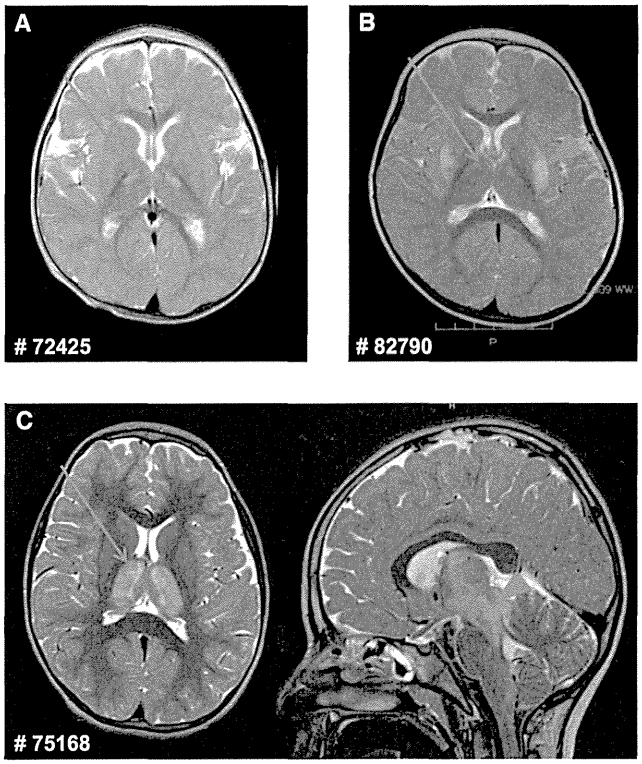

Figure 2. Brain MRI of Affected Individuals #72425, #82790, and #75168

(A) MRI of individual #72425 shows small T2 hyperintensities in the anterior thalamus bilaterally (arrow).

(B) In individual #82790, T2-weighted MRI shows bilateral hyperintensities in the putamen (arrowhead) and weakly also in the anterior thalamus (arrow).

(C) T2-weighted MRI of individual #75168 shows pronounced bilateral hyperintensities affecting the whole thalamus (arrow, axial view at the left) and extending to the mesencephalon (arrowhead, sagittal view at the right).

remained stable, without cardiac symptoms or arrhythmia. At 7 months of age, she had cardiogenic shock and metabolic acidosis. Heart ultrasound detected dilated cardiomyopathy and decreased contractility (ejection fraction 35%). She presented hyperlactatemia (20 mmol/l), hyperalaninemia (1,175 μmol/l; normal range, 190–450 μmol/l), and an increased lactate-to-pyruvate ratio (47; normal range, 10–20). Her disorder progressed despite intensive medication for heart failure. She died at the age of 9 months of cardiac insufficiency with arrhythmia.

Her younger sister, individual #83905 (family F9, Figure 1A), had a very similar clinical picture. She died at 6 months of age of cardiac insufficiency unresponsive to resuscitation procedures.

Genetic, biochemical, and clinical findings are summarized in Table 1. Pedigrees of the families we studied are shown in Figure 1A. The location of the identified mutations within the gene and the conservation of the affected amino acid (aa) residues are shown in Figure 1B. Individual #49665 (F1: II-2) was found to carry a frame shift and one missense variant. The next generation sequencing (NGS) data demonstrated a compound heterozygous status of the two variants (Figure S1 available online). Individual #76671 (F5: II-2) was homozygous for an intronic single base pair deletion, c.665−2delA, which is predicted to cause the loss of a splice acceptor site. Analysis of cDNA from fibroblasts revealed a shorter transcript, and sequencing found that in more than 95% of transcripts, the downstream acceptor of exon 7 was used for splicing, resulting in the skipping of exon 6 including the conserved G1-box guanine nucleotide-binding signature motif (Figure S2). Individual #82790 (F8: II-2) was found to be compound heterozygous for a missense mutation c.8G>T (p.Arg3Leu) and a 24 bp deletion c.934_957del (p.Gly312_Val319del). The 24 bp deletion is predicted to cause the deletion of 8 amino acids containing conserved residues. The p.Arg3Leu substitution at the very N terminus of the protein is scored as a predicted polymorphism but causes a loss of a positively charged residue, which is predicted to interfere with mitochondrial targeting (Predotar, PsortII). The two missense variants found in individual #66654, c.[673G>A; 964G>C], p.[Glu225Lys; Ala322Pro], were identical to the variants found on the paternal allele of individual #72425 (F3: II-1). Analysis of parental DNA revealed that both variants were also located on the same allele in individual #66654, meaning that only one allele is affected. Because of this observation, combined with the absence of a heart phenotype and because this individual is the only one exhibiting an isolated complex I defect, we consider the mutations found in *GTPBP3* not to be causative in subject #66654.

In summary, the identification of 13 different alleles in 11 individuals with suspected mitochondrial disease from 9 families provides strong evidence for the pathological role of mutant *GTPBP3* in the investigated families. It links *GTPBP3* mutations to combined respiratory chain complex deficiency (9/11), cardiomyopathy (9/11), lactic acidosis (11/11), and encephalopathy (4/11).

Brain MRI was performed in three individuals (Figure 2). It showed bilateral T2 hyperintensities in the thalamus, ranging from weak (#82790) or small (#72425) changes in the anterior thalamus to very pronounced hyperintensities affecting the whole thalamus in individual #75168. In addition, T2 hyperintensities affected the putamen bilaterally in individual #82790 and extended markedly to the mesencephalon in individual #75168. Taken together, the MRI involvement of basal ganglia and brainstem resembles the (MRI) findings in Leigh syndrome (which is, however, an ill-defined entity).

Skin fibroblast cell lines were available from seven individuals for functional studies. We first analyzed the cellular oxygen consumption rate (OCR^{13}) by microscale respirometry with the XF96 extracellular flux analyzer (Seahorse Bioscience). When cells of individuals from families F1 to F5 were cultured in glucose-containing medium, only cell lines from individuals #75191 (F4: II-4) and #76671 (F5: II-2) showed a decreased OCR (of 59% and 58%, respectively) indicating defective oxidative phosphorylation

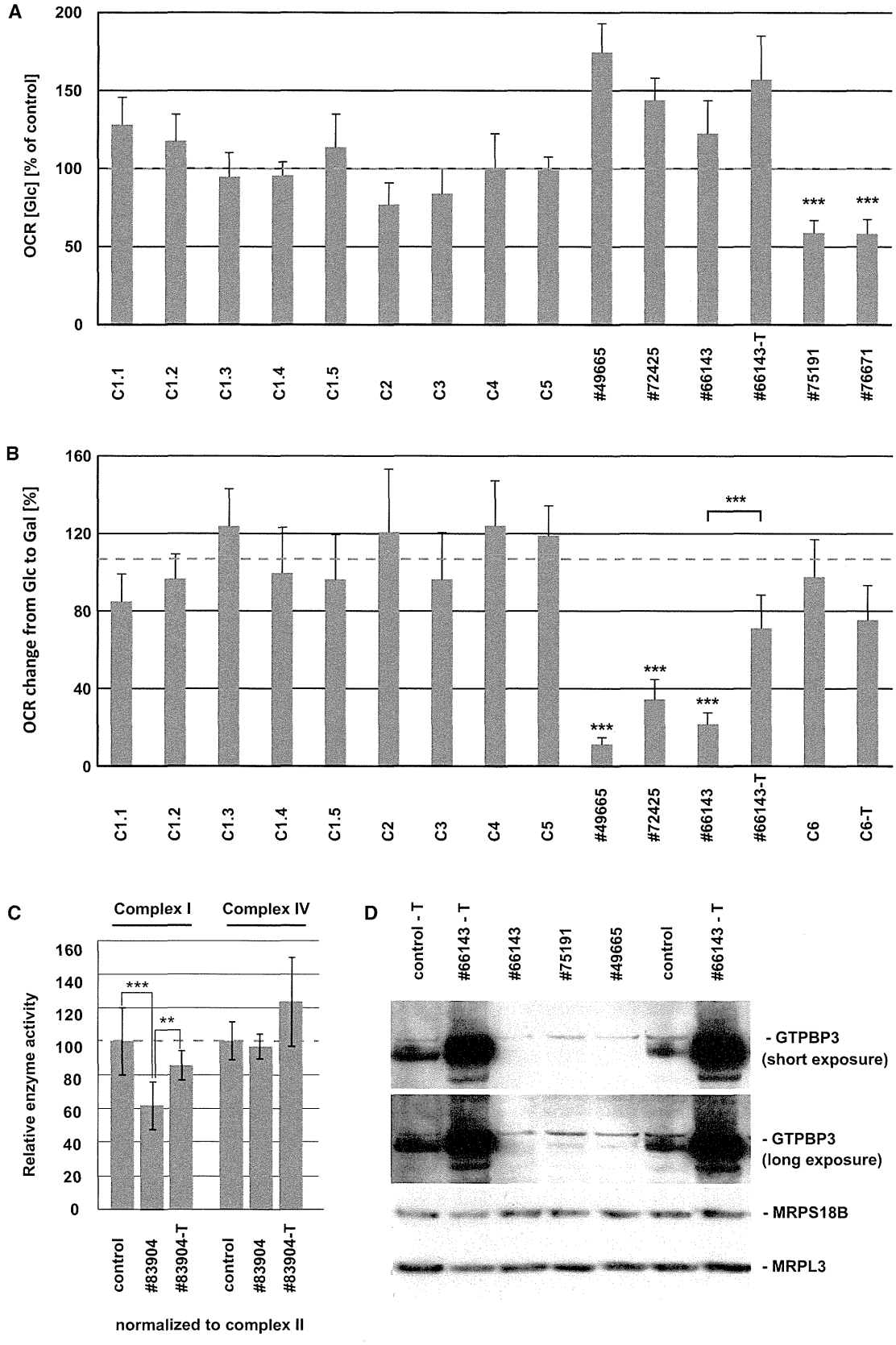

Figure 3. Analysis of Respiration Defects and GTPBP3 Protein Levels in Fibroblast Cell Lines

(A) Oxygen consumption rate (OCR) of fibroblast cell lines from five affected individuals and five control subjects cultured in high-glucose (Glc) medium. Each analysis was performed in more than 15 replicates. Control one (C1) was measured five time at different passage numbers (C1.1–1.5, NHDFneo, Lonza). OCR was expressed as percentage relative to the average of all controls. Cells from

(legend continued on next page)

(Figure 3A). When cells were cultured with galactose as the primary carbon source, rather than glucose, cells are forced to rely on oxidative phosphorylation rather than glycolysis in order to meet the energy demand.[14,15] Accordingly, in control cells an increase in OCR of approximately 2-fold was observed when galactose was substituted as the primary carbon source. This increase in OCR was impaired in fibroblasts from affected individuals #49665 (F1: II-2), #66143 (F2: II-2), and #72425 (F3: II-1), which showed OCR increases of only 11%, 35%, and 22%, respectively (Figure 3B). In order to confirm that defects in GTPBP3 are the cause of this defect, we transduced three cell lines with a wild-type copy of *GTPBP3* cDNA (RefSeq NM_32620.3) by using a lentiviral vector (pLenti 6.3/V5 TOPO, Life Technologies) as previously described.[16,17] Fibroblasts from individuals #49665 and #66143 were used for the rescue experiment, with fibroblasts from #66654 (subject with only one affected allele) being included as a control (C6). Unfortunately, we were unable to recover any viable cells from subject #49665 after the transduction procedure. Although the transduction had no noticeable effect on the control cell line (C6-T), transduced fibroblasts from #66143 (#66143-T) displayed a significant improvement of OCR in galactose-containing medium (Figure 3B). Furthermore, we detected an isolated respiratory chain complex (RCC) I deficiency in a fibroblast cell line from family 9. Cotransfection of individual #83904 fibroblasts with two putative *GTPBP3* isoforms amplified by RT-PCR, RefSeq NM_32620.3 and NM_0128855.2 (missing 63 base pairs of exon 8), significantly improved enzyme activities of RCC I (pIRES2-EGFP, Clontech) (Figure 3C). Analysis of the protein levels of GTPBP3 in five fibroblast cell lines demonstrated reduced or undetectable amounts in individuals #49665, #75191, #66143, #83904, and #83905, although they showed a clear increase after transduction or transfection (Figures S4 and 3D). In conclusion, our data demonstrate a causal role for *GTPBP3* mutations in the oxidative metabolism deficiency in these individuals.

Given that homologs of GTPBP3 in other systems have been implicated in protein synthesis, we next concentrated on the analysis of GTPBP3 in mitochondrial translation. The synthesis of mtDNA-encoded polypeptides, investigated by pulse-labeling of mitochondrial translation products via [^{35}S]methionine in fibroblasts of affected individuals (for methods see Haack et al.[18]) was severely and uniformly decreased to 20%–30% of control levels in individuals #49665, #66143, and #75191 (Figures 4A and 4B). There was no detectable defect in fibroblasts from individual #72425, which might be explained by the relatively low conservation of the mutated residue in this individual (Figure 1B). In order to exclude possible defects of mitochondrial transcription or precursor RNA processing, we analyzed all mitochondrially encoded rRNAs and mRNAs in fibroblasts of individuals #49665, #66143, #72425, and #75191 by RNA blotting and by RNA-seq in fibroblasts of individual #49665. We found no differences in the expression levels of the mt-RNAs between case and control subjects. On average, the mt-RNA expression levels were only 6% lower in individual #49665 as compared to control individuals (data not shown). We did not observe any appreciable reduction in steady-state levels of mature RNAs, nor was there any accumulation of precursor RNAs (Figure S3A). Next, we analyzed the steady-state levels of mt-tRNAs, including those five species for which the τm^5 U modification has been reported in mammals (Gln, Glu, Lys, Leu^{UUR}, and Trp).[4] We again observed no appreciable changes in their steady-state levels (Figure S3B). In order to further corroborate a direct role of GTPBP3 in mitochondrial translation, we downregulated its expression via RNA interference in HeLa cells (Figure 4C). Reduction of GTPBP3 protein levels upon RNAi treatment of HeLa cells was comparable to the reduction of its level in *GTPBP3* mutant fibroblasts (Figure 4D). Downregulation of *GTPBP3* expression resulted in a general mitochondrial translation defect, similarly to the reduction observed in subject fibroblasts (Figure 4D). In conclusion, the reduced translation efficiency observed in three out of four *GTPBP3* mutant cell lines, as well as in human cells treated with *GTPBP3* RNAi, confirmed an important function for GTPBP3 in efficient mitochondrial protein synthesis.

In order to test the consequences of this reduced translation rate upon the protein levels of OXPHOS complexes in mutant fibroblast cell lines, we analyzed the steady-state levels of several nuclear-encoded subunits of the OXPHOS system by immunoblotting. In fibroblasts from individuals #72425, #75191, and #76671 (F3: II-1, F4: II-4, and F5: II-2), we observed strongly reduced amounts of RCC IV. Fibroblasts from subjects #72425, #75191, and #49665 also showed reduced levels of RCC I, whereas the levels

individuals #75191 and #76671 showed a significant reduction of oxygen consumption whereas cells from individuals #49665, #72425, and #66143 presented no defective respiration. Error bar indicates 1 SD; ***$p < 0.001$.

(B) Oxygen consumption rate of fibroblast cells cultured in galactose (Gal) growth medium. The average increase of OCR from five control cells cultured in galactose-containing medium compared to glucose-containing medium was 107%. Cell lines from individuals #49665, #72425, and #66143 show significant lower increase in OCR. Lentiviral expression of wt*GTPBP3* in cells from individual #66143 significantly increases the change in OCR although it has only little effect in control cells (C6-T). Error bar indicates 1 SD; ***$p < 0.001$.

(C) Activities of respiratory chain complexes I and IV (expressed as ratio to CII activity) are decreased in individual #83904 cells transfected by electroporation with empty vector (pIRES2-EGFP) according to the manufacturer's protocol (LONZA) but are improved upon expression of *GTPBP3* cDNAs from the same plasmid. Measurements were performed as previously described.[29,30] Error bar indicates 1 SD. Activity in controls was set as 100%. **$p < 0.01$, **$p < 0.001$.

(D) Levels of GTPBP3 were reduced in cells from individuals #49665, #75191, and #66143 and elevated after transduction with wt*GTPBP3* cDNA. MRPS18B and MRPL3 served as mitochondrial loading controls.

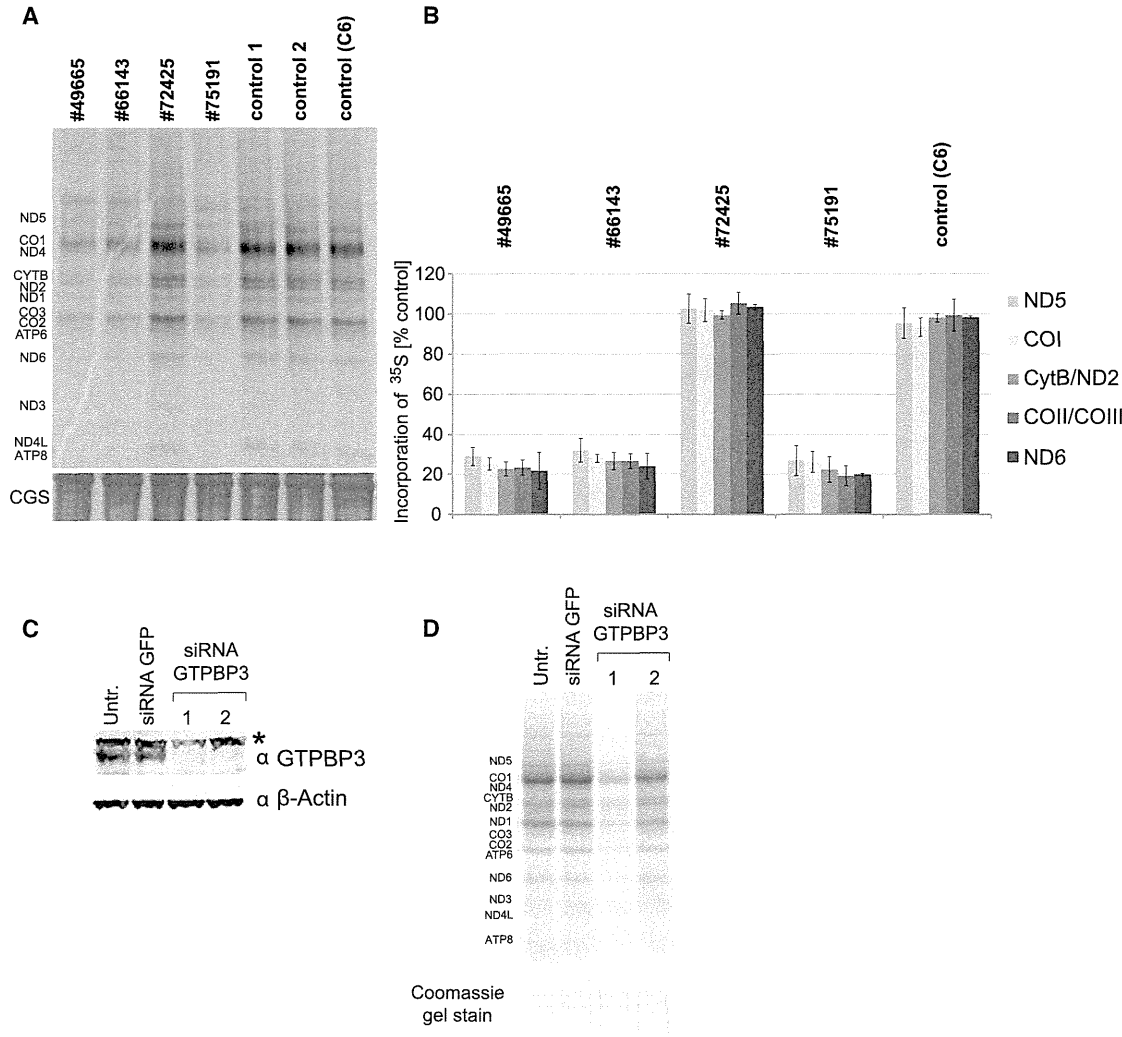

Figure 4. Analysis of Mitochondrial Protein Synthesis in Primary Fibroblasts and in HeLa Cells Treated with RNAi against GTPBP3
(A) [^{35}S]methionine metabolic labeling of mitochondrial proteins in fibroblasts. Products of mitochondrial translation were labeled with [^{35}S]methionine for 30 min, separated by a 4%–12% gradient SDS-PAGE, and visualized by autoradiography. To validate equal protein loading, a small section of the gel was stained with Coomassie (CGS). Fibroblasts from individuals #49665, #66143, and #75191 demonstrate significant inhibition of mitochondrial protein synthesis although translation in cells from individual #72425 is not affected.
(B) Quantification of radiolabelled products of mitochondrial translation. Incorporation of [^{35}S] as in (A) was quantified by ImageQuant software after exposure to a PhosphorImager screen from three independent experiments. Error bar indicates 1 SD.
(C) Downregulation of GTPBP3 in HeLa cells via RNA interference. Immunoblot analysis of total HeLa cell lysate transfected with two different siRNA to GTPBP3 show decreased level of GTPBP3 upon RNAi treatment for 6 days. siRNA to GFP was used as transfection control. Asterisk indicates nonspecific band recognized by anti-GTPBP3 antibody in HeLa cells. β-actin serves as a loading control. Two different siRNA duplexes targeting GTPBP3 were used, 1 and 2.
(D) Mitochondrial translation in HeLa cells upon GTPBP3 downregulation. HeLa cells were transfected for 6 days with siRNA against GTPBP3 and subjected to [^{35}S]methionine metabolic labeling. Inactivation of GTPBP3 leads to the reduced efficiency of mitochondrial translation. Two different siRNA duplexes targeting GTPBP3 were used, 1 and 2.

of RCC II and V remained normal in all cell lines (Figure 5). The diminished steady-state levels of respiratory chain complexes I and IV in fibroblast cell lines are in agreement with the impaired mitochondrial de novo translation rates in these cells and match the enzymatic defects identified in muscle biopsies of the same individuals.

Within an international cooperation between European (Germany, UK, Italy, France, and Belgium), Israeli, and Japanese Centers for mitochondrial disorders, we provide statistically convincing evidence for *GTPBP3* mutations leading to mitochondrial disease. To further support collaborative studies, the global mitochondrial disease community has established a Mitochondrial Disease Sequence Data Resource (MSeqDR) for common genomic data deposition and mining.

The genotype-driven analysis performed here was independent from the clinical presentation. Nevertheless, we identified common clinical features of the affected individuals that include lactic acidosis (11/11), cardiomyopathy (9/11), and neurological symptoms (6/11). The latter

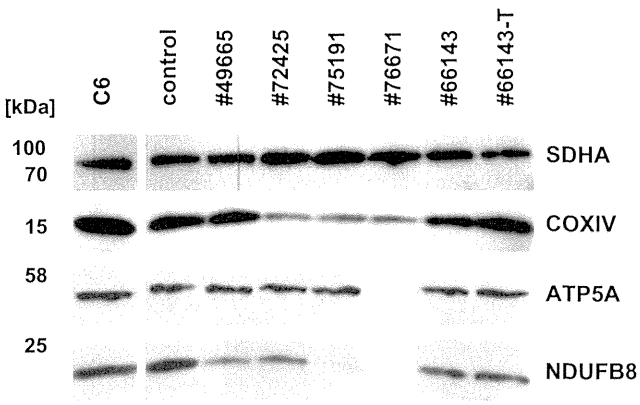

Figure 5. Immunoblot Analysis of OXPHOS Proteins in Fibroblasts

10 μg of detergent-solubilized total cell extract was subjected to immunoblot analysis of OXPHOS components. Amounts of SDHA (complex II) and ATP5A (ATPase) were unchanged in all individuals. In cells from individuals #72425, #75191, and #76671, a reduction of COXIV (complex IV) was observed. Cells from individuals #49665, #72425, and #75191 showed decreased levels of NDUFB8 (complex I). Antibodies used: mouse antibodies against SDHA (ab14715), NDUFB8 (ab110242), ATP5A (ab14748), and rabbit antibodies against COXIV (ab16056) from Abcam and rabbit anti GTPBP3 (HPA042158) from SIGMA Aldrich.

comprised symptoms such as development delay, intellectual disability, feeding difficulties, muscle hypotonia, fatigue, visual impairment, and epileptic seizures. Severity of the disease ranged from neonatal onset and death to late-infantile onset and survival into the second decade of life. Most affected individuals, however, manifested clinical symptoms before their first birthday. This is consistent with the normal cellular respiration, in organello translation, and normal levels of respiratory chain complexes reported in individuals less severely affected and the significantly reduced mitochondrial translation, respiration, and low levels of complex I and IV in those severely affected.

Modifications of the tRNA "wobble-base" in the anticodon loop are required for accurate and efficient codon recognition. The modification of position 5 (xm^5) of the U34 wobble-base of certain tRNAs is evolutionarily well conserved, although different modified side chains have been identified in different species. In mammals, mitochondria 5-taurinomethyluridine (τm^5U34) is found at the wobble-base position.[19] Based upon studies in bacteria and yeast mitochondria, GTPBP3 and MTO1 have been proposed to generate this modification in mammalian mitochondria. Although this prediction awaits direct biochemical validation, the proposed functional conservation of GTPBP3 and MTO1 has been supported by the mitochondrial localization of these proteins in human cells and by complementation of the respiratory-deficient phenotype in yeast by their mammalian homolog cDNAs.[20,21] Functional deficiency of homologs of GTPBP3 and MTO1 in bacteria and yeast mitochondria has been associated with abnormal U34 modification and consequently a reduced efficiency of translation.[21–23] Our data support an analogous activity of GTPBP3 in human mitochondria since we identified a reduced efficiency of translation in three cell lines with *GTPBP3* mutations and in cells with RNAi-mediated downregulation of GTPBP3 expression. Other groups have also reported impaired protein synthesis and reduced mitochondrial function in GTPBP3-depleted cells.[24] The defect in mitochondrial translation was a likely cause of the combined respiratory chain complex deficiency detected in muscle tissues of all but one affected individual.

Like *GTPBP3* mutations, *MTO1* mutations are also associated with hypertrophic cardiomyopathy (HCM), lactic acidosis, and combined respiratory chain deficiency. An association of *MTO1* mutations with impaired mitochondrial translation has yet to be shown for human mitochondria, but the common clinical presentation provides support for a common pathomechanism in the U34 modification for both diseases. So far, all individuals with *MTO1* mutations presented a HCM. However, nearly all of them have been specifically screened for *MTO1* mutations based on the clinical presentation of a HCM. Clinical and MRI signs of brain involvement are found for both *GTPBP3* and *MTO1* cases. The genotype-driven investigation presented here identified individuals with lactic acidosis, developmental delay, and MRI involvement of thalamus, putamen, and brainstem but without HCM. It can be expected that the clinical spectrum associated with MTO1 deficiency will also broaden, with more subjects being genome-wide investigated. In a very recent study, Taylor et al. indeed reported a case subject with *MTO1* mutations and central neurological features who did not have a cardiomyopathy.[25]

Our study highlights that defects in mitochondrial translation, probably owing to incorrect posttranscriptional modification of mt-tRNAs, are an important contributory factor to the spectrum of human mitochondrial disease. Recent data have suggested that more than 7% of all mt-tRNA residues undergo posttranscriptional modification, with close to 30 different modifications so far described.[4] Therefore, it is expected that future WES analyses of individuals clinically diagnosed with mitochondriopathy will reveal further mutations within genes coding for mt-tRNA modifiers. Indeed, in addition to the aforementioned mutations in *MTO1* and *TRMU*, mutations in *PUS1* (MIM 608109) (which introduces pseudouridine [Ψ] at base positions 27, 28, and 29 in several mt-tRNAs) have been reported in subjects affected with mitochondrial myopathy and sideroblastic anemia (MLASA)[26] (MIM 600462) and very recent studies have identified mutations in *TRIT1* (which is responsible for i^6A37 modification of a subset of mt-tRNAs) in individuals with severe combined mitochondrial respiratory chain defects.[27] Furthermore, mtDNA mutations in mt-tRNA genes, which are a very frequent cause of human respiratory chain deficiencies (MITOMAP), might also affect mt-tRNA modification. Related to the present study, it has been reported that τm^5U34 is not present in mt-tRNALeu^{UUR} harboring the m.3243A>G mutation (or other pathological mutations) responsible for mitochondrial encephalopathy, lactic

acidosis, and stroke-like episodes (MELAS) (MIM 540000). The absence of τm^5U34 has been suggested to be responsible for the mitochondrial translation defect in these subjects.[28] These results imply that deficiency of mt-tRNA modification plays a critical role in the molecular pathogenesis of human respiratory chain disease. Further studies of these pathways, such as analysis of tissue-specific regulation of mt-tRNA-modifying enzymes, might help to explain the clinical heterogeneity observed for mitochondrial diseases caused by mutations in mt-tRNA genes.

In conclusion, this study shows a mitochondrial translation disorder with a broad spectrum of clinical presentations, which emphasizes the importance of post-transcriptional modification of mitochondrial tRNAs for proper mitochondrial function.

Supplemental Data

Supplemental Data include four figures and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2014.10.017.

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Web Resources

The URLs for data presented herein are as follows:

MITOMAP, http://www.mitomap.org/MITOMAP
MSeqDR, https://mseqdr.org/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
Predotar, https://urgi.versailles.inra.fr/predotar/predotar.html
PSORTII Prediction, http://psort.hgc.jp/form2.html
RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq

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Supplemental Data

Mutations in *GTPBP3* Cause a Mitochondrial Translation Defect Associated with Hypertrophic Cardiomyopathy, Lactic Acidosis, and Encephalopathy

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Figure S1

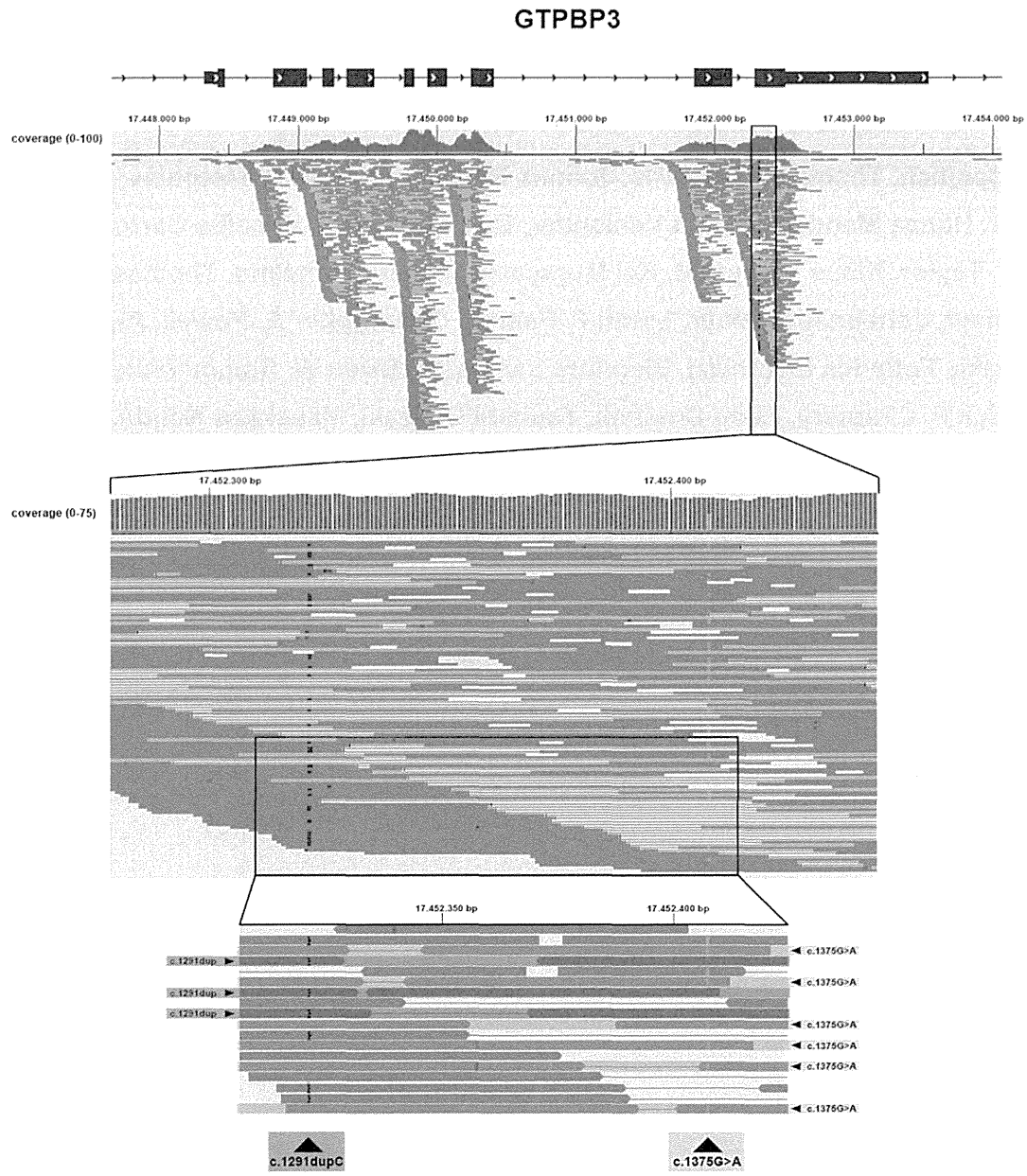

Figure S1) Segregation analysis in family F1 in WES data

The two mutations identified in family F1 (c.1291dupC and c.1375G>A) are only separated by 97 bp which allowed analysis of both alleles despite the lack of parental material. 13 paired sequence reads were identified which covered the region of both variants. All reads contained either of the two mutations demonstrating a compound heterozygous status of the two variants. Figure S1 shows three sequence reads containing the c.1291dupC variant and five reads containing the c.1375G>A variant.

Figure S2

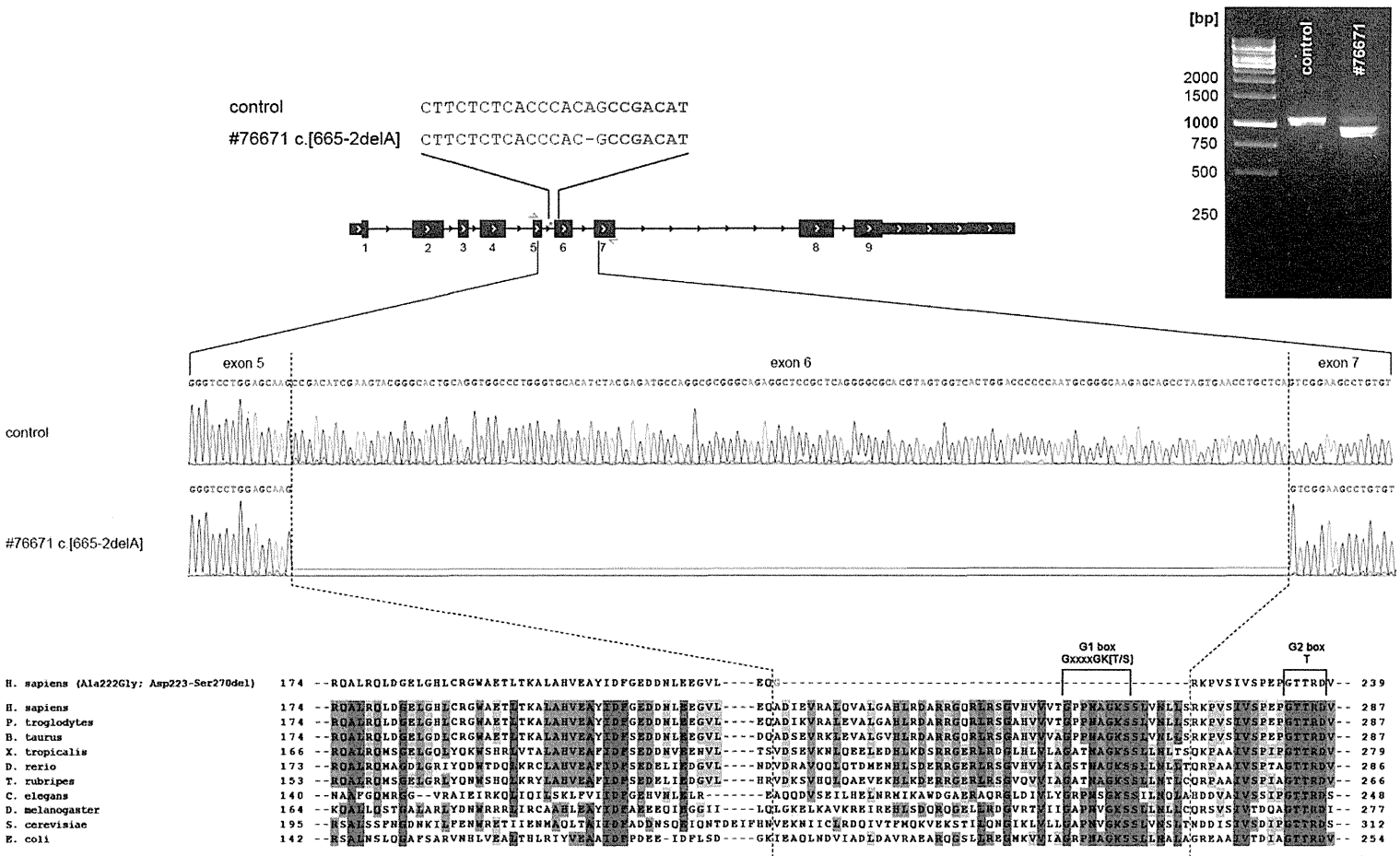

Figure S2) Splice site mutation in individual #76671 causes skipping of exon 6

Analysis of cDNA derived from fibroblasts of individual #76671 yielded a smaller than expected PCR product, indicating alternative splicing. Sanger sequencing revealed that the c.665-2delA mutation affects the conserved splice acceptor site. The splice accepto upstream of exon 7 is alternatively used, yielding a mature mRNA that lacks exon 6. The resulting protein product is predicted tc contain a 1 amino acid exchange followed by a 48 amino acid deletion (p.Ala322Gly; Asp223-270del).

Figure S3

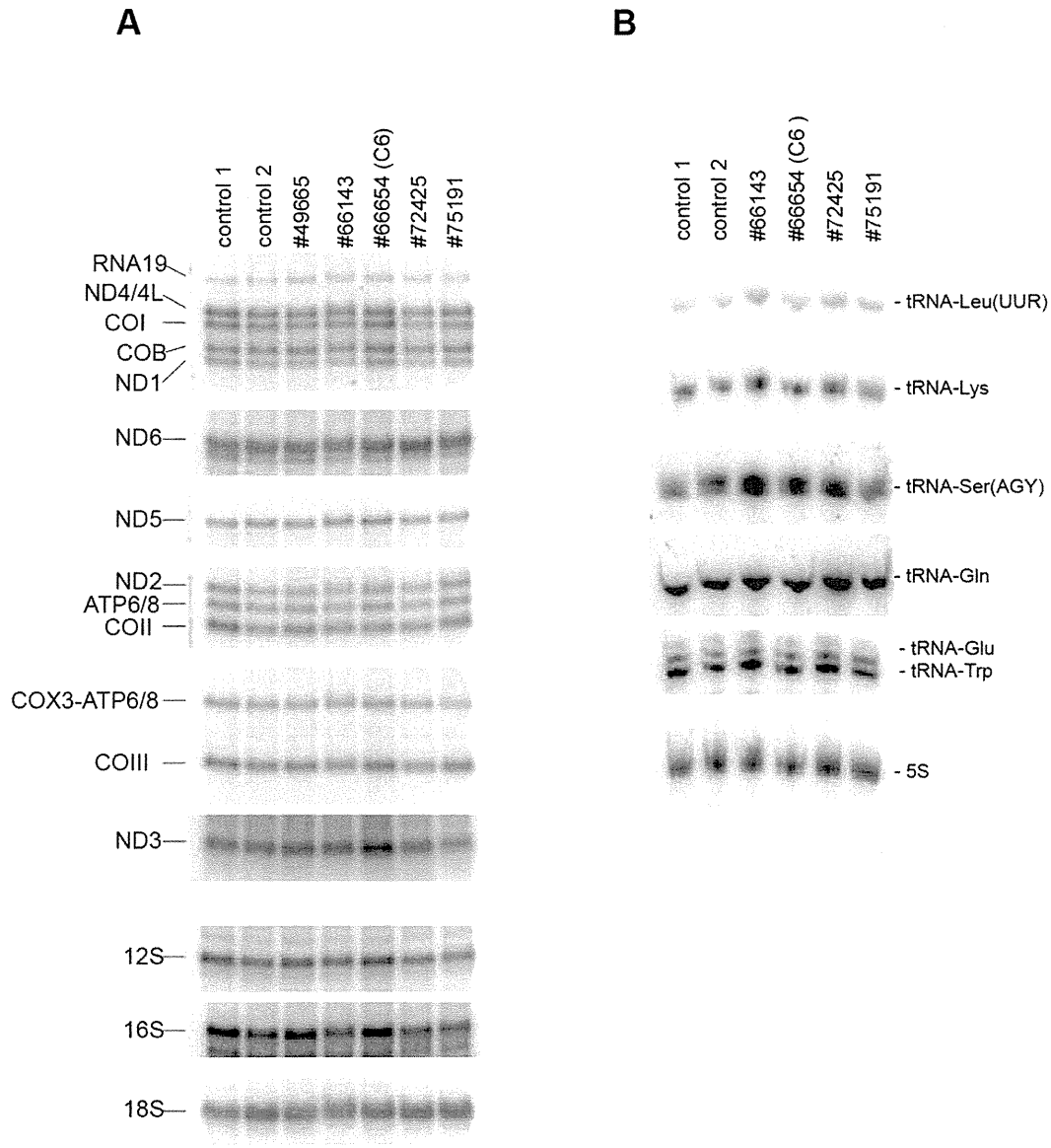

Figure S3) Northern blot analysis of the steady-state levels of mitochondrial transcripts in GTPBP3 patient fibroblasts.

A) Northern blot analysis of total RNA isolated from the *GTPBP3* patient or control primary fibroblasts. The blots were probed with the mt-mRNA- and mt-rRNA-specific probes as indicated. The cytosolic 18S rRNA was used as a loading control.

B)) High-resolution Northern blot analysis of total RNA isolated from the *GTPBP3* patient or control primary fibroblasts. The blots were probed with the mt-tRNA -specific probes as indicated. The cytosolic 5S rRNA was used as a loading control.

Figure S4

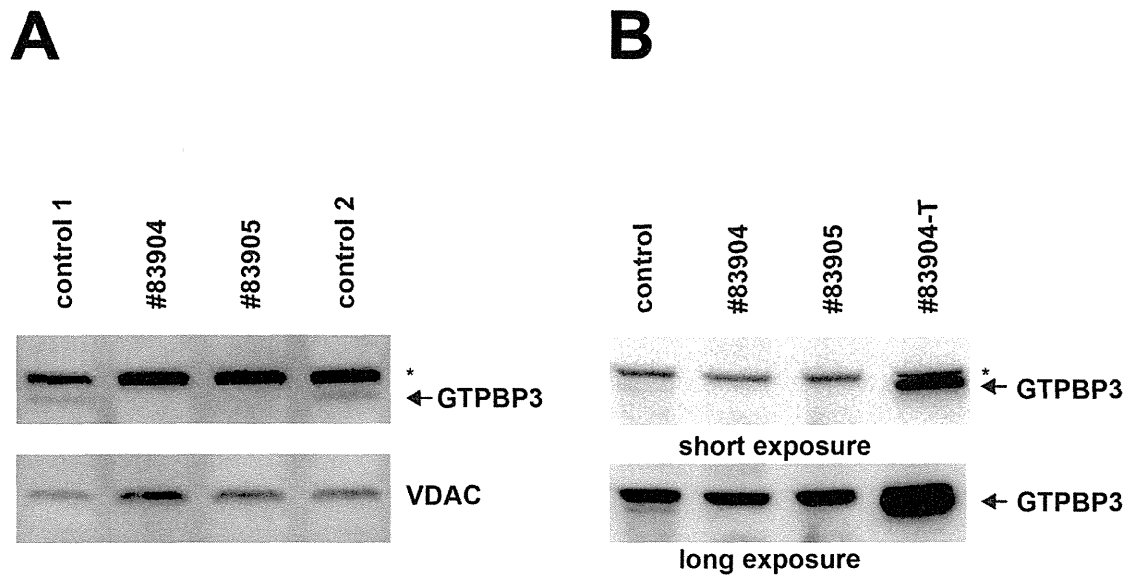

Figure S4) Analysis of GTPBP3 protein levels in patient fibroblasts

A) Immunoblot analysis of GTPBP3 protein levels in fibroblasts from affected individuals #83904 and #83905 from family F9. VDAC served as a mitochondrial loading control. (Asterics indicates a non-specific band.)

B) Comparison of the electrophoretic migration of GTPBP3 in un-transfected cells (lane control) and cells derived from one of the affected individuals transfected with a plasmid (pIRES2-EGFP) for *GTPBP3* cDNA expression (lane #83904-T). (Asterics indicates a non-specific band.)

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Clinical Observations

Fever of Unknown Origin as the Initial Manifestation of Valproate-Induced Fanconi Syndrome

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ABSTRACT

BACKGROUND: Valproate-induced Fanconi syndrome is a rare adverse effect of valproate. Severely disabled patients who require tube feeding are reported to be susceptible to valproate-induced Fanconi syndrome. Although most patients with valproate-induced Fanconi syndrome are asymptomatic and detected incidentally with findings such as hypophosphatemia, hypouricemia, increased urinary β2-microglobulin, and generalized hyperaminoaciduria, clinical symptoms such as bone fracture, fever, tachypnea, and edema have been reported. **PATIENT DESCRIPTION:** This 15-year-old, severely disabled, tube-fed, male patient with cytochrome oxidase deficiency had taken valproate for 3 years when he developed fever for 3 weeks. Hypophosphatemia, hypouricemia, hypokalemia, increased urinary β2-microglobulin, and generalized hyperaminoaciduria, as well as hypocarnitinemia, were found, indicating that he had Fanconi syndrome. Valproate was the most likely cause of Fanconi syndrome in this patient. After discontinuation of valproate, the fever resolved immediately, and the laboratory findings normalized. **CONCLUSION:** Valproate-induced Fanconi syndrome should be considered when individuals taking valproate develop fever of unknown origin.

Keywords: Fanconi syndrome, valproate, fever of unknown origin, side effects, valproate-induced Fanconi syndrome

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Introduction

Fanconi syndrome is a generalized dysfunction of the proximal renal tubules that causes urinary excretion of amino acids, glucose, phosphate, bicarbonate, uric acid, and other substances. Valproate (VPA)-induced Fanconi syndrome is a rare adverse effect of VPA.[1] Several case reports have shown that severely disabled, tube-fed patients are vulnerable to VPA-induced Fanconi syndrome.[2,3] Most patients with VPA-induced Fanconi syndrome are diagnosed during routine or incidental laboratory examinations without any obvious symptoms.[2] The case of a severely disabled, tube-fed patient with cytochrome oxidase deficiency who presented with fever of unknown origin that was most likely caused by VPA-induced Fanconi syndrome is presented.

Patient Description

This 15-year-old boy was born at 41 weeks' gestation with a birth weight of 3270 g. His Apgar score was 3 at 1 minute and 6 at 5 minutes. He developed spastic tetraplegia and needed tube feeding. At age 4 months, he presented with infantile spasms. He was diagnosed with probable Leigh syndrome because of high lactate levels in the blood (39.6 mg/dL) and cerebrospinal fluid (34.5 mg/dL), as well as high-intensity signals in bilateral basal ganglia and thalami on T2-weighted magnetic resonance imaging at age 2 years. Pyruvate dehydrogenase complex activities in the lymphocytes and respiratory chain complex activities in the muscle, as well as histopathology of the skeletal muscle, were normal. Screening for known mitochondrial DNA mutations was negative. Seizure control was poor, and he had been on VPA and carbamazepine without l-carnitine supplementation from age 12 years. Two

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months before his admission at age 15 years, the VPA dose was increased (34 mg/kg/day) because of seizure deterioration, and l-carnitine (6 mg/kg/day) was added as a supplement to prevent secondary carnitine deficiency.

He was admitted to our hospital because of high-grade fever (39.1°C) that had lasted for 3 days. Bronchial pneumonia was suspected, and cefotaxime was administered. However, the fever persisted for 3 weeks. His body weight decreased from 16.7 kg at admission to 14.8 kg during the 3 weeks despite sufficient water (1800 mL/day) and caloric intake (1500 kcal/day). The following laboratory examinations were normal: white blood cell count, C-reactive protein, serological tests for viral or mycoplasma infection, antinuclear antibodies, rheumatoid factor, thyroid hormones, lymphocyte stimulation test for VPA, and bacterial and mycotic cultures. However, the erythrocyte sedimentation rate was high (90 mm/hr). Whole-body [F^{18}]-fluorodeoxyglucose positron emission tomography scan did not reveal any findings indicating focal inflammation. On the other hand, hypophosphatemia (1.3 mg/dL; normal 2.6-6.3 mg/dL), hypokalemia (3.3 mEq/L; normal 3.6-5.2 mEq/L), and hypouricemia (1.6 mg/dL; normal 2.0-7.0 mg/dL) were found, indicating the possibility of proximal renal tubular dysfunction. Urinalysis showed proteinuria, glycosuria, elevated β2-microglobulin (4460 μg/L; normal <230 μg/L), and generalized hyperaminoaciduria. These results confirmed Fanconi syndrome. In addition, hypocarnitinemia (19.0 μmol/L) was found despite carnitine supplementation, indicating secondary carnitine deficiency due either to VPA or Fanconi syndrome, or both. VPA was discontinued at 18 days from the start of fever. Four days later, the high fever resolved, and he gained 1 kg of weight. After the fever resolved, the l-carnitine supplement was increased (40 mg/kg/day) to treat hypocarnitinemia. Two months later, laboratory findings associated with Fanconi syndrome were normal. After recovery, a skin biopsy revealed cytochrome oxidase deficiency. The patient was not rechallenged with VPA, and no recurrence of Fanconi syndrome has been evident for more than 2 years.

Discussion

Fanconi syndrome is characterized by a general dysfunction of proximal renal tubules that causes urinary excretion of amino acids, glucose, phosphate, bicarbonate, uric acid, and other substances.[4] Recently, VPA-induced Fanconi syndrome has been recognized mostly in severely disabled patients.[2,3] The fever of unknown origin and weight loss in the present patient were likely the manifestations of VPA-induced Fanconi syndrome due to (1) the patient's vulnerability to this condition due to his diagnosis of cytochrome oxidase deficiency and severe disability requiring tube feeding, and (2) the fact that fever of unknown origin, weight loss, and laboratory findings indicative of Fanconi syndrome normalized after discontinuation of VPA.

A search of the PubMed database and the Japan Medical Abstract Society website for articles using the keywords "Fanconi syndrome" and "valproate" or "valproic acid" identified 20 reports of 49 patients (Table).[1-20] In these, sex and age were described in 37 patients (19 males and 18 females), with ages ranging from 2 to 32 years (median age, 8 years). As previously reported,[3] a high percentage of Japanese patients (36 of 49 patients) was evident. Among the 49 patients identified, 47 were described as being severely disabled (n = 42) or not (n = 5); in addition, feeding was described in 41 of the 49 patients, with 36 reported as being tube-fed. The duration of VPA treatment ranged from 3 months to 21 years (median, 4 years), and the VPA blood levels ranged from 21 to 141 μg/mL (median, 77.6 μg/mL). When VPA was discontinued, 45 of 47 patients recovered completely from VPA-induced Fanconi syndrome.

The duration needed for recovery ranged from 1 week to 18 months (median, 4 months). Two patients developed renal failure or continuing proteinuria despite the discontinuation of VPA.[6,13] Thus, the clinical course of VPA-induced Fanconi syndrome in the present patient was similar to the previous reports. Among the 13 patients in whom serum carnitine levels or carnitine supplementation was described, 3 patients had hypocarnitinemia,[11,16,19] 1 patient had a normal carnitine level,[15] and 9 patients with no description of serum carnitine levels had carnitine supplementation.[12,13,17,18] None of the patients whose serum carnitine levels were described had fever of unknown origin. Thus, there was no apparent association between hypocarnitinemia and prolonged fever, as appeared in the present patient. Furthermore, the weight loss and elevated erythrocyte sedimentation rate found in the present patient were not described in the other reported patients with VPA-induced Fanconi syndrome.

Overall, in 19 of the 49 reported patients, VPA-induced Fanconi syndrome was found on routine or incidental laboratory examinations without any obvious symptoms. In the remaining 30 patients, the initial clinical manifestations led to the diagnosis: 11 had fracture, 9 had fever, 3 had tachypnea, 2 had edema, 2 had weakness, 1 had anorexia, abdominal pain, and myopathy-like symptoms, 1 had hypertension, and 1 had fatigue and confusion. Among the 9 patients with fever,[8,9,14,15,20] 2 had prolonged fever described as fever of unknown origin.[8] In the remaining 7 patients, VPA-induced Fanconi syndrome was diagnosed and treated before the fever became prolonged.

In the present case, it took time to suspect VPA-induced Fanconi syndrome because fever is a common symptom in severely disabled patients. After infection was ruled out, more time was taken to rule out various other causes. The present case report, therefore, suggests the importance of considering VPA-induced Fanconi syndrome in severely disabled patients on VPA who develop prolonged fever and of measuring serum phosphate, uric acid, and electrolyte levels, as well as urinalysis including β2-microglobulin. The present case also suggested that supplementation with less than 10 mg/kg of carnitine is insufficient in these patients.

Although the precise pathogenic mechanism of VPA-induced Fanconi syndrome remains unknown, a mitochondrial abnormality in the proximal renal tubules is a typical finding of drug-induced Fanconi syndrome. There are at least three hypotheses for the pathogenesis of the renal tubular dysfunction. The first is an inhibition of β-oxidation in the mitochondria of the proximal renal tubules either directly by VPA or indirectly by the secondary carnitine deficiency caused by VPA.[1,5] The second is tubulo-interstitial nephritis (TIN) caused by hypersensitivity to VPA or a direct toxic effect of VPA.[5,8] The third is increased oxidative stress due to the VPA-induced decrease in plasma glutathione peroxidase activity, which causes mitochondrial dysfunction in the tubules.[1] The mechanisms of fever in VPA-induced Fanconi syndrome are unknown. Only two reported patients had prolonged fever of unknown origin, and TIN was found in one patient.[8] Because TIN can cause fever and weight loss,[8] fever of unknown origin and weight loss in the present patient might have been caused by TIN, but it could not be confirmed without renal biopsy. Another possibility is

TABLE.
Previously Published Cases of VPA-induced Fanconi Syndrome

Patient number	Age (y)/ Sex	Clinical remarks	Severe disability	Tube feeding	VPA duration	VPA blood level, μg/mL	Other AEDs	Time to recovery	Opportunity that disclosed FS	Reference
1	27/F	Epilepsy	No	No	5 y	136	None	1 wk	Fatigue, confusion	1
2	6/M	Cerebral palsy	Yes	Yes	5 1/2 y	81.4	PHT, CLB	6 mo	Edema	2
3	15/M	Cerebral palsy	Yes	Yes	14 1/2 y	75	CBZ, CLB	3 mo	Laboratory study	2
4	10/F	Cerebral palsy, lissencephaly	Yes	-	16 mo	69.8	PB, GBP	5 mo	Laboratory study	2
5	9/M	Anoxic encephalopathy	Yes	Yes	Several years	49.3	PB, DZP	4 mo	Laboratory study	2
6	4/F	Perinatal anoxic encephalopathy	Yes	Yes	3 1/2 y	60.2	CBZ	4 mo	Laboratory study	2
7	8/M	West syndrome	Yes	-	7 y	64	CZP, TPM	2 mo	Laboratory study	3
8	6/F	Cerebral retardation	Yes	-	2 1/2 y	-	CLB	14 mo	Weakness	3
9	12/M	Petit mal epilepsy	No	No	19 mo	-	PB	6 mo	abdominal pain, myopathy-like	4
10	10/M	Severe and global neurolgic impairment	Yes	-	10 mo	-	PHT, lorazepam	3 mo	Hypertension	5
11	10/M	Epilepsy	No	No	18 mo	-	None	Renal failure	Laboratory study	6
12	9.5/M	Birth asphyxia	-	-	8 y	-	CZP, CBZ	4 mo	Fracture	7
13	19/M	Cerebral palsy	-	-	-	-	PB	-	Fracture	7
14	15/M	Near-drowning	Yes	Yes	13 y	89.2	None	3 mo	Laboratory study	8
15	6/F	Neonatal asphyxia	Yes	Yes	6 y	73.9	CLB	2 mo	Laboratory study	8
16	6/F	Neonatal asphyxia	Yes	Yes	6 y	119.8	None	2 mo	Laboratory study	8
17	2/F	Early infantile epileptic encephalopathy	Yes	Yes	12 mo	94.7	ZNS, CLB	3 mo	Fever of unknown origin	8
18	4/M	Pachygyria	Yes	Yes	4 y	62	None	18 mo	Laboratory study	8
19	8/F	Neonatal asphyxia	Yes	Yes	8 y	95	None	2 mo	Laboratory study	8
20	13/F	Neonatal asphyxia	Yes	Yes	7 y	141	None	12 mo	Fever of unknown origin	8
21	8/F	Chromosome abnormality	Yes	Yes	6 y	98.96	-	2 mo	fever	9
22	4/M	West syndrome	Yes	Yes	3 y	40	PB, ZNS	9 mo	Laboratory study	10
23	10/M	Partial epilepsy	No	No	12 mo	21	None	18 mo	Laboratory study	10
24	7/M	Lissencephaly	Yes	Yes	7 y	129.7	PB, CZP	2 mo	Edema	10
25	7/F	Near-drowning	Yes	Yes	2 y	57.2	CBZ	15 mo	Laboratory study	10
26	9/F	Hypoxic ischemic encephalopathy	Yes	Yes	8 y	81.6	-	4 mo	Laboratory study	11
27	22/M	West syndrome	Yes	Yes	21 y	-	-	1 mo	Laboratory study	12
28	-	Encephalopathy sequelae	Yes	Yes	2 y	-	ZNS	3 mo	Fracture	13
29	-	Encephalopathy sequelae	Yes	Yes	4 y	-	ZNS	3 mo	Fracture	13
30	-	Chromosome abnormality	Yes	Yes	4 y	-	CBZ, PHT	Proteinuria	Laboratory study	13
31	-	Epilepsy	Yes	Yes	3 y	-	KBr	1 mo	Laboratory study	13
32	-	Brain malformation	Yes	Yes	9 y	-	PB	3 mo	Fracture	13
33	-	Cerebral palsy	Yes	Yes	4 y	-	ZNS	6 mo	Tachypnea	13
34	-	Cerebral palsy	Yes	Yes	8 y	-	none	9 mo	Fracture	13
35	-	Cerebral palsy	Yes	Yes	3 y	-	CLB, ZNS, PB, KBr	9 mo	Fracture	13
36	-	Neurodegenerative disease	Yes	Yes	3 y	-	CZP, PB, DZP	6 mo	Fracture	13
37	-	Cerebral palsy	Yes	Yes	3 y	-	ZNS, PB	2 mo	Fracture	13
38	-	Encephalopathy sequelae	Yes	Yes	3 mo	-	ZNS, PB	6 mo	Tachypnea	13
39	-	Neurocutaneous syndrome	Yes	Yes	12 mo	-	ZNS	6 mo	Tachypnea	13
40	4/M	Congenital myopathy	Yes	Yes	-	-	None	3 mo	Lower respiratory tract infection	14
41	8/F	West syndrome	Yes	Yes	6 y	74.2	PHT, PB, CZP, ZNS	5 mo	Pneumonia	15
42	2/F	Chromosome abnormality	Yes	Yes	2 y	122.7	CBZ, CLB	2 mo	Gastroenteritis	15
43	3/M	Epilepsy	Yes	Yes	3 y	65.6	ZNS, GBP	1 mo	Upper respiratory infection	15
44	8/F	Myoclonic epilepsy	Yes	Yes	7 y	-	CLB	12 mo	Fracture	16
45	14/M	Epilepsy	No	No	2 y	-	-	6 mo	Weakness	17
46	10/F	Partial deletion of chromosome 4p	Yes	Yes	9 y	-	TPM	12 mo	Laboratory study	18
47	2/F	Type 2 Gaucher disease	Yes	Yes	2 y	80.2	CLB, TPM	2 mo	Fracture	19
48	11/M	Cerebral palsy, epilepsy	Yes	-	11 y	-	PB, ZNS CLB, KBr	A couple of weeks	Respiratory illness	20
49	32/F	Neonatal asphyxia	Yes	-	-	-	-	-	Pneumonia	20

Abbreviations:
AEDs = Antiepileptic drugs
CBZ = Carbamazepine
CLB = Clobazam
CZP = Clonazepam
DZP = Diazepam
FS = Fanconi syndrome
GBP = Gabapentin
KBr = Potassium bromide
PB = Phenobarbital
PHT = Phenytoin
TPM = Topiramate
VPA = Valproate
ZNS = Zonisamide
Severe disability means bedridden or wheelchair-bound.

uncoupling or "loose coupling" of oxidative phosphorylation in the mitochondria, which may cause fever and weight loss because of hypermetabolism, as found in Luft's disease.[21] However, valproate is not known to have an uncoupling effect. Our patient had a cytochrome oxidase deficiency, which itself could cause Fanconi syndrome. However, the fact that Fanconi syndrome resolved with VPA withdrawal indicated that the mitochondrial disease was not a direct cause of Fanconi syndrome in the present patient.

In conclusion, VPA-induced Fanconi syndrome should be considered when patients taking VPA develop fever of unknown origin. Furthermore, individuals taking VPA, especially those who are severely disabled and tube-fed, should be given carnitine supplementation and be periodically screened for Fanconi syndrome.

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COQ4 Mutations Cause a Broad Spectrum of Mitochondrial Disorders Associated with CoQ_{10} Deficiency

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Primary coenzyme Q10 (CoQ_{10}) deficiencies are rare, clinically heterogeneous disorders caused by mutations in several genes encoding proteins involved in CoQ_{10} biosynthesis. CoQ_{10} is an essential component of the electron transport chain (ETC), where it shuttles electrons from complex I or II to complex III. By whole-exome sequencing, we identified five individuals carrying biallelic mutations in *COQ4*. The precise function of human COQ4 is not known, but it seems to play a structural role in stabilizing a multiheteromeric complex that contains most of the CoQ_{10} biosynthetic enzymes. The clinical phenotypes of the five subjects varied widely, but four had a prenatal or perinatal onset with early fatal outcome. Two unrelated individuals presented with severe hypotonia, bradycardia, respiratory insufficiency, and heart failure; two sisters showed antenatal cerebellar hypoplasia, neonatal respiratory-distress syndrome, and epileptic encephalopathy. The fifth subject had an early-onset but slowly progressive clinical course dominated by neurological deterioration with hardly any involvement of other organs. All available specimens from affected subjects showed reduced amounts of CoQ_{10} and often displayed a decrease in CoQ_{10}-dependent ETC complex activities. The pathogenic role of all identified mutations was experimentally validated in a recombinant yeast model; oxidative growth, strongly impaired in strains lacking COQ4, was corrected by expression of human wild-type *COQ4* cDNA but failed to be corrected by expression of *COQ4* cDNAs with any of the mutations identified in affected subjects. *COQ4* mutations are responsible for early-onset mitochondrial diseases with heterogeneous clinical presentations and associated with CoQ10 deficiency.

Coenzyme Q (CoQ), or ubiquinone, is a lipophilic component of the electron transport chain (ETC), where it shuttles electrons derived from NADH and $FADH_2$ to ETC complex III (cIII) or ubiquinone-cytochrome c reductase. The main electron donors to CoQ are ETC complexes I (cI) and II (cII) but also include other mitochondrial flavoproteins, for instance, electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial (ETF-dehydrogenase [ETFDH]), which is the terminal component of fatty acid β-oxidation and branched-chain amino acid oxidation pathways. CoQ can also act as an antioxidant and a membrane stabilizer, is a cofactor of additional mitochondrial enzymes (e.g., uncoupling protein UCP1),[1,2] and plays an indispensable role in the de novo pyrimidine biosynthesis as the electron acceptor from dihydroorotate dehydrogenase.[3–5]

CoQ is a 1,4-benzoquinone with a tail of 10 isoprenyl units in humans (CoQ_{10}) but of variable length in other species (e.g., CoQ_6 in yeast). The synthesis of the isoprenoid moieties proceeds via either mevalonate or

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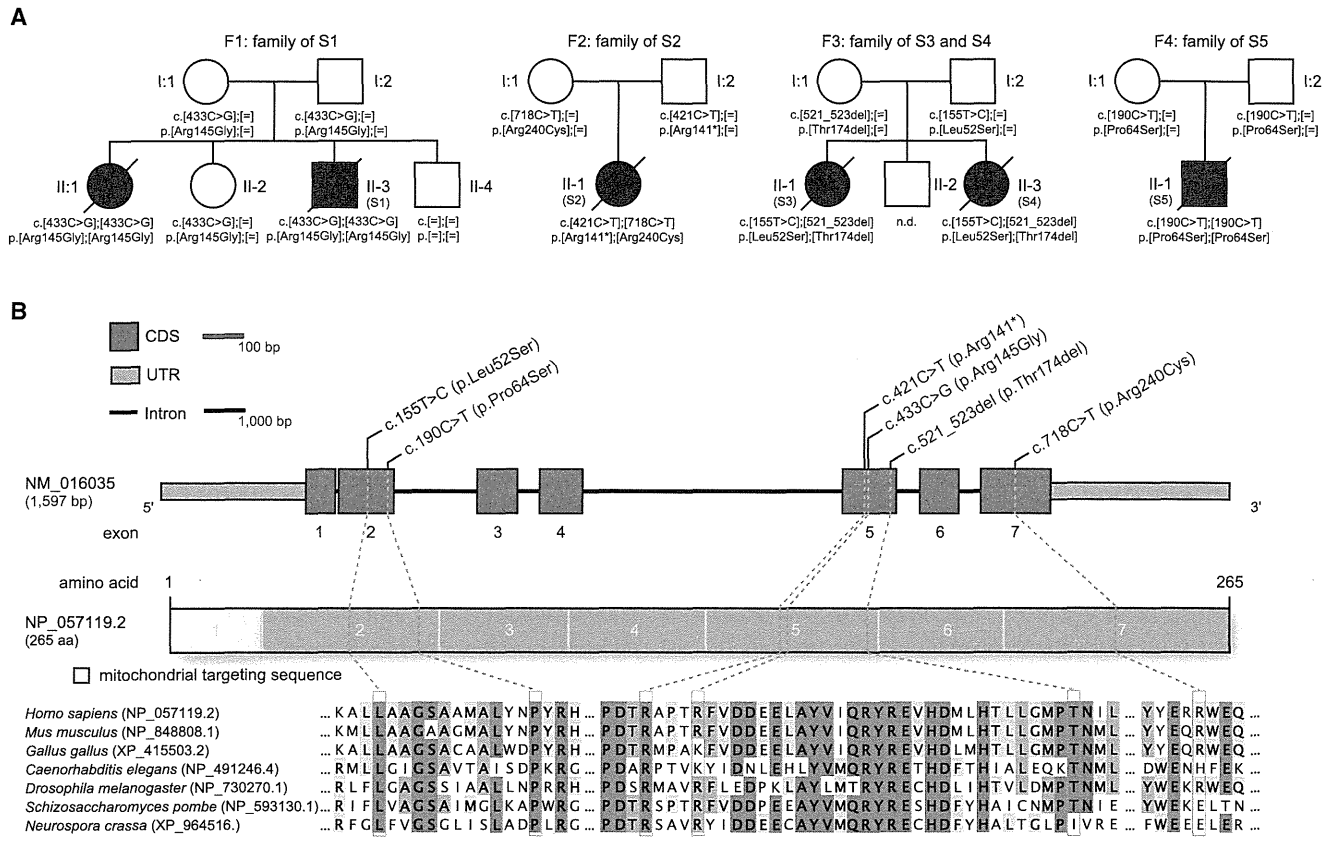

Figure 1. Pedigrees of Investigated Families and *COQ4* Structure and Conservation of Identified Mutations
(A) Pedigrees of four families affected by mutations in *COQ4*. The mutation status of affected and unaffected family members is indicated by closed and open symbols, respectively.
(B) *COQ4* structure showing the identified mutations. The structure of the gene product, COQ4, is also shown with known domains and localization and conservation of amino acid residues affected by the mutations. Intronic regions are not drawn to scale.

2-C-methyl-D-erythritol 4-phosphate pathways, whereas the aromatic precursor of the CoQ benzoquinone ring is p-hydroxybenzoate, derived from tyrosine.[6] After the isoprenoid "tail" is bound to the aromatic "head," the ring undergoes sequential modification. At least ten enzymes participate in CoQ biosynthesis; in yeast, and possibly mammals as well, these enzymes are all localized in mitochondria.

Primary CoQ_{10} deficiency is the biochemical signature of a group of rare, clinically heterogeneous autosomal-recessive disorders caused by mutations in several genes encoding proteins involved in CoQ_{10} biosynthesis.[7] Mutations in *COQ2* (MIM 609825), *COQ6* (MIM 614647), *ADCK3* (*COQ8* [MIM 606980]), *ADCK4* (MIM 615573), *COQ9* (MIM 612837), *PDSS1* (MIM 607429), and *PDSS2* (MIM 610564) have been reported in subjects with severe infantile mitochondrial syndromes associated with severe tissue CoQ_{10} deficiency, whereas the genetic bases underpinning adult-onset CoQ_{10} deficiency remain mostly undefined.[8,9] *COQ4* (MIM 612898) codes for a ubiquitously expressed 265-amino-acid protein that is peripherally associated with the mitochondrial inner membrane on the matrix side;[10] the precise function of human COQ4 is not known, but the yeast ortholog seems to play a structural role crucial in the stabilization of a multiheteromeric complex including several, if not all, of the CoQ biosynthetic enzymes.[11]

We report here the identification of pathogenic biallelic *COQ4* mutations in a total of five individuals from four families; these subjects were part of a cohort of severe mitochondrial cases where the CoQ_{10} defect was not anticipated. The family pedigrees are shown in Figure 1A.

Subject 1 (S1; II-3, family 1), a boy, was the third of four siblings and was born to healthy, non-consanguineous Italian parents after an uncomplicated pregnancy and elective cesarean delivery. His oldest sister (II-1), who presented with bradycardia and hypotonia, died at birth, and his 16-year-old second sister and his 5-year-old brother are alive and well. At birth, S1 had a weight of 3,410 g, a length of 49.5 cm, and a head circumference of 34.5 cm. Apgar scores were 7 and 10 at 1 and 5 min after birth, respectively. At birth, his condition appeared critical, given that he showed severe hypotonia, areflexia, acrocyanosis, bradycardia, and respiratory insufficiency. Ultrasound examination revealed markedly decreased motility of the left ventricle with an ejection fraction of 20%–25%. No evidence of hepatic or renal impairment was observed. Dobutamine infusion via an umbilical venous catheter was