Mitochondrial EFTs defects in juvenile-onset Leigh disease, ataxia, neuropathy, and optic atrophy

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ABSTRACT

Objective: We report novel defects of mitochondrial translation elongation factor Ts (EFTs), with high carrier frequency in Finland and expand the manifestations of this disease group from infantile cardiomyopathy to juvenile neuropathy/encephalopathy disorders.

Methods: DNA analysis, whole-exome analysis, protein biochemistry, and protein modeling.

Results: We used whole-exome sequencing to find the genetic cause of infantile-onset mitochondrial cardiomyopathy, progressing to juvenile-onset Leigh syndrome, neuropathy, and optic atrophy in 2 siblings. We found novel compound heterozygous mutations, c.944G>A [p.C315Y] and c.856C>T [p.Q286X], in the *TSFM* gene encoding mitochondrial EFTs. The same p.Q286X variant was found as compound heterozygous with a splice site change in a patient from a second family, with juvenile-onset optic atrophy, peripheral neuropathy, and ataxia. Our molecular modeling predicted the coding-region mutations to cause protein instability, which was experimentally confirmed in cultured patient cells, with mitochondrial translation defect and lacking EFTs. Only a single *TSFM* mutation has been previously described in different populations, leading to an infantile fatal multisystem disorder with cardiomyopathy. Sequence data from 35,000 Finnish population controls indicated that the heterozygous carrier frequency of p.Q286X change was exceptionally high in Finland, 1:80, but no homozygotes were found in the population, in our mitochondrial disease patient collection, or in an intrauterine fetal death material, suggesting early developmental lethality of the homozygotes.

Conclusions: We show that in addition to early-onset cardiomyopathy, *TSFM* mutations should be considered in childhood and juvenile encephalopathies with optic and/or peripheral neuropathy, ataxia, or Leigh disease. ***Neurology*® 2014;83:743–751**

GLOSSARY

cDNA = complementary DNA; **EFTs** = elongation factor Ts; **EFTu** = elongation factor Tu; **GDP** = guanosine diphosphate; **RC** = respiratory chain; **SISu** = Sequencing Initiative Suomi project; **SNP** = single nucleotide polymorphism; **tRNA** = transfer RNA; **TSFM** = Ts translation elongation factor, mitochondrial.

Mitochondrial respiratory chain (RC) dysfunction is a major cause of metabolic disorders in adults and children. Mutations in mitochondrial and nuclear genes encoding mitochondrial protein synthesis machinery have been reported to cause an exceptionally variable spectrum of manifestations, despite all resulting in RC defects.[1] The vast majority of identified mitochondrial translation defects originate from mutations in mitochondrial DNA–encoded transfer RNAs (tRNAs), but a growing number of nuclear genes are being identified. Mutations have been found in genes encoding elongation factors mtEFG1, EFTs, and elongation factor Tu (EFTu),[2–6] mitoribosomal proteins (MRPL44, MRPS16, MRPS22, MRPL3, MRPL12),[7–11] aminoacyl-tRNA synthetases,[12–20] tRNA-modifying enzyme TRMU,[21] pseudouridine synthase 1 PUS1,[22] peptide release factor C12orf65,[23] and RNase ELAC2.[24] The complexity of the mitochondrial translation system suggests that the identified factors are only the tip of the iceberg.[25,26]

Supplemental data at Neurology.org

Next-generation sequencing methodology, in particular whole-exome sequencing, has shown to be an efficient way to find new disease-causing mutations in mitochondrial patients with variable clinical manifestations.[3] Here, we applied exome sequencing to find the genetic cause of infantile-onset cardiomyopathy and Leigh disease, with slowly progressive, multiorgan RC deficiency, and report novel pathogenic mutations in the *TSFM* gene encoding for mitochondrial elongation factor Ts (EFTs).

METHODS Standard protocol approvals, registrations, and patient consents. All samples were taken according to the Declaration of Helsinki, with informed consent. The study was approved by the ethical review board of the Helsinki University Central Hospital and The Hospital District of Southwest Finland.

Exome sequencing. The patients' genomic DNA was isolated from cultured fibroblasts (P1) or from muscle biopsy tissue (P3) and used for NimbleGen Sequence Capture 2.1M Human Exome v1.0 Array (Roche NimbleGen, Madison, WI) and sequenced with the Illumina Genome Analyzer-IIx platform (Illumina Inc., San Diego, CA) as previously described in reference 27. The identified single nucleotide polymorphisms (SNPs) were then filtered using dbSNP133, 1000 Genomes, and MitoCarta.[28]

Determination of the frequency of identified sequence variants. Carrier frequencies were determined with 1000 Genomes, NHLBI (National Heart, Lung and Blood Institute Exome Variant Server) SNP, or dbSNP133 (National Center for Biotechnology Information) databases. Furthermore, because the patients originated from Finland, we utilized the exome data collection of more than 3,000 Finnish individuals from the Sequencing Initiative Suomi project (SISu; described in http://sisu.fimm.fi) and further characterized the carrier frequency of the p.Q286X variant by genotyping in a total of 32,497 subjects from the Finnish population (Finnish cohorts, http://sisu.fimm.fi).

The presence of the identified *TSFM* mutations in patients with mitochondrial disease was determined utilizing the data collection of 76 exomes in the laboratory of A.S.

Intrauterine fetal death samples were collected in Turku University Central Hospital between the years 2001 and 2011, excluding all early neonatal deaths, multiple pregnancies, and intrapartum deaths. The relevant conditions leading to intrauterine fetal death were classified according to the newly developed pathophysiologic classification system (ReCoDe, Relevant Condition of Death).[29] DNA was extracted from paraffin blocks of 32 tissue samples by using NucleoSpin FFPE DNA kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. A 120–base pair amplification product was sequenced using the primers 5′-ctggacgatgagcctggg-3′ and 5′-ccctgaggctgcacatact-3′.

RNA analysis. RNA was extracted with a standard Trizol and chloroform method from cultured fibroblasts of P1, P2, and one control, and from cultured myoblast of P3 and 2 controls with nonmitochondrial disease. Complementary DNA (cDNA) was synthesized using manufacturer's instructions (Maxima; Fermentas/Thermo Scientific, Pittsburgh, PA). The *TSFM* transcript levels were analyzed in 3 experimental replicates by real-time quantitative PCR with SYBR Green (Thermo Scientific) using the following primers: 5′-ggtgtttatcgcggctagag-3′ and 5′-ccacaagtctccagagctttc-3′ for *TSFM* and 5′-cgctctctgctcctcctgtt-3′

and 5′-ccatggtgtctgagcgatgt-3′ for *GAPDH*. The complete *TSFM* cDNA was PCR-amplified and sequenced with primers 5′-ggtgtttatcgcggctagag-3′ and 5′-ctcggtctgaagaggtttgg-3′. For solid-phase minisequencing, primers 5′-ccttaaggcacttttggaaat-3′ and 5′-B-ctcggtctgaagaggtttgg-3′ (B, biotinylated) and 5′-ggcagagactaagatgctgtcc-3′ (for Q286X mutation) were used with ³H-dCTP or ³H-dTTP, and radioactivity counts were measured by using the 2450 MicroBeta² microplate counter (PerkinElmer, Waltham, MA).[30]

Protein modeling. Structure modeling for human elongation factors EFTu and EFTs was based on resolved structures of bovine homologs (PDB id 1XB2, chain A for modeling EFTu and chain B for EFTs).[31] Human and bovine EFTs sequences share 83.8% identity and 88.2% similarity, while EFTu sequences share 94.5% and 96.5%, respectively (identity matrix BLOSUM62). The following resources were used: structure modeling, SWISS-Model Server (http://swissmodel.expasy.org/), alignment mode; protein sequences of eukaryotic and prokaryotic EFTu and EFTs, UniProt knowledgebase (http://www.uniprot.org/); multiple sequence alignments, PROMALS3D (http://prodata.swmed.edu/promals3d/promals3d.php); assembly of EFTu-EFTs complex, structure analysis of the attained model and figure preparation, Discovery Studio V.2.5.5 (Accelrys).

Protein amount and translation analyses. For Westerns, 15 μg of mitochondrial protein extracts were separated in 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. We used antibodies against SDH-70 kDa (MitoSciences, Eugene, OR) and EFTs/Tu (polyclonal, kindly provided by Linda Spremulli, Chapel Hill, NC). Experiments were done 3 times. Pulse translation assay was performed with 3 replicates as described previously.[32] Total cellular proteins (30 μg) were separated in 12% to 20% polyacrylamide-gradient gel, which was imaged on a PhosphorImager plate (GE Healthcare, Waukesha, WI), analyzed with a Typhoon analyzer and ImageQuant software (Amersham Biosciences, Piscataway, NJ). As a control for fibroblast translation defect, we used patient fibroblasts with homozygous, previously published p.R312W mutation in EFTs (a kind gift from Professor Thorburn).[3]

RESULTS Clinical findings. Table 1 summarizes the clinical findings of the patients. Patient 1 (P1, II-3, family 1) (figure 1A), a female, is the third child of nonconsanguineous Finnish parents. Her brother (II-2) died suddenly at the age of 1 year during respiratory syncytial virus infection and was diagnosed postmortem to have cardiomyopathy. The early milestones of P1 were normal. At the age of 10 months, she was diagnosed with dilated cardiomyopathy. She had a severe episode of cardiac insufficiency during a respiratory infection at 1 year of age and was scheduled for cardiac transplantation, but this plan was canceled a few years later because the cardiomyopathy was no longer progressive. Her cardiac situation has remained stable on medication.

On examination at the age of 1 year, she had elevated serum and CSF lactate values. The skeletal muscle sample revealed combined deficiency of RC. Her brain MRI and early cognitive and motor development were normal. A cognitive decline manifested during the first school years, and brain MRI at the

Table 1	Clinical presentation of the patients		
Characteristics	**Patients**		
	P1 (sister of P2)	P2 (sister of P1)	P3
Sex	F	F	M
Age at onset	10 mo	16 mo	15 y
Current age	21 y	15 y	21 y
Cardiomyopathy (age at onset)	Hypertrophic (10 mo)	Hypertrophic (16 mo)	—
Myopathy			
Clinical symptoms	Weakness, fatigue	Mild weakness	—
EMG (age)	Some polyphasic potentials (21 y)	Mild myopathic (13 y)	Normal (15 y)
Peripheral neuropathy			
Severity and type	Mild, axonal sensorimotor	Mild, axonal sensorimotor	Moderate, axonal sensorimotor
Electroneurography lower limbs (age)	MCV normal, sensory amplitude decreased, absent F-responses (21 y)	Motor and sensory amplitude decreased (13 y)	MCV decreased, no responses from sural nerve (15 y)
Electroneurography upper limbs (age)	Normal (21 y)	Normal (13 y)	SCV and sensory amplitude decreased (15 y)
Nerve biopsy (age)	ND	ND	Moderate axon loss, mild hypertrophy, and demyelination (16 y)
Optic atrophy (age at onset)	+ (teenage)	+ (teenage)	+ (teenage)
Cognitive function	Decline (7 y)	Mild learning difficulties	Normal
Lactate			
In blood (ref. 0.6–2.4 mmol/L)	5.5	2.7	Normal (0.9)
In CSF (ref. <2.8 mmol/L)	3.0	ND	ND
Brain MRI	Normal (3 y), Leigh (9 y)	Normal (11 y)	Normal (15 y)
Muscle			
Histopathology (age)	COX-negative fibers (1 y)	Normal (1 y)	Atrophic type-II fibers, COX-deficient fibers (16 y)
RC enzyme activities (age)	CI + III 32%, CIV 45% (1 y)	CIV 18% (1 y)	Normal (16 y)
mtDNA depletion/deletions	−/−	ND	−/−
Additional findings	Dystonic dyskinesia	—	Ataxia

Abbreviations: C = complex; COX = cytochrome c oxidase; MCV = motor conduction velocity; mtDNA = mitochondrial DNA; ND = not done; RC = respiratory chain; ref. = reference; SCV = sensory conduction velocity.
Symbols: + = present; − = absent.

age of 9 years showed bilateral signal intensity in putamen and globus pallidus (in T2-weighted images), consistent with Leigh syndrome. Optic atrophy manifested at teenage and progressed to severe visual impairment. At present, at 21 years of age, she has frequent dystonic movements in her face, neck, and shoulder region, and mild peripheral axonal sensorimotor neuropathy.

Patient 2 (P2, II-4, family 1) (figure 1A), sister of P1, is a 15-year-old girl with hypertrophic cardiomyopathy diagnosed at the age of 16 months. Her cardiomyopathy has been stable on medication. She has mild learning difficulties, mild optic atrophy, and mild peripheral axonal sensorimotor neuropathy. Brain MRI at the age of 11 years was normal.

Patient 3 (P3, II-1, family 2) (figure 1A), a male, was born at full term to healthy Finnish parents. His development was normal until the age of 15 years when he experienced subacute deterioration of vision and was diagnosed with optic neuropathy. MRI of the brain was normal. He had mild tremor in the trunk and limbs, difficulties in keeping balance, and absent Achilles tendon reflexes. Electroneuromyography showed peripheral axonal neuropathy of mixed type. Histologic analysis of a sural nerve sample revealed a moderate loss of myelinated axons with a mild tendency to hypertrophy and demyelination. At the age of 21 years, the patient has ataxia and optical and peripheral neuropathy, but no cardiac symptoms.

Identification of *TSFM* mutations by whole-exome sequencing. We determined the sequence of the whole exome of P1 by next-generation sequencing technology. Sequencing coverage was at 96.47% of

Figure 1 Exome sequencing revealed 3 novel mutations in the *TSFM* gene in 2 families
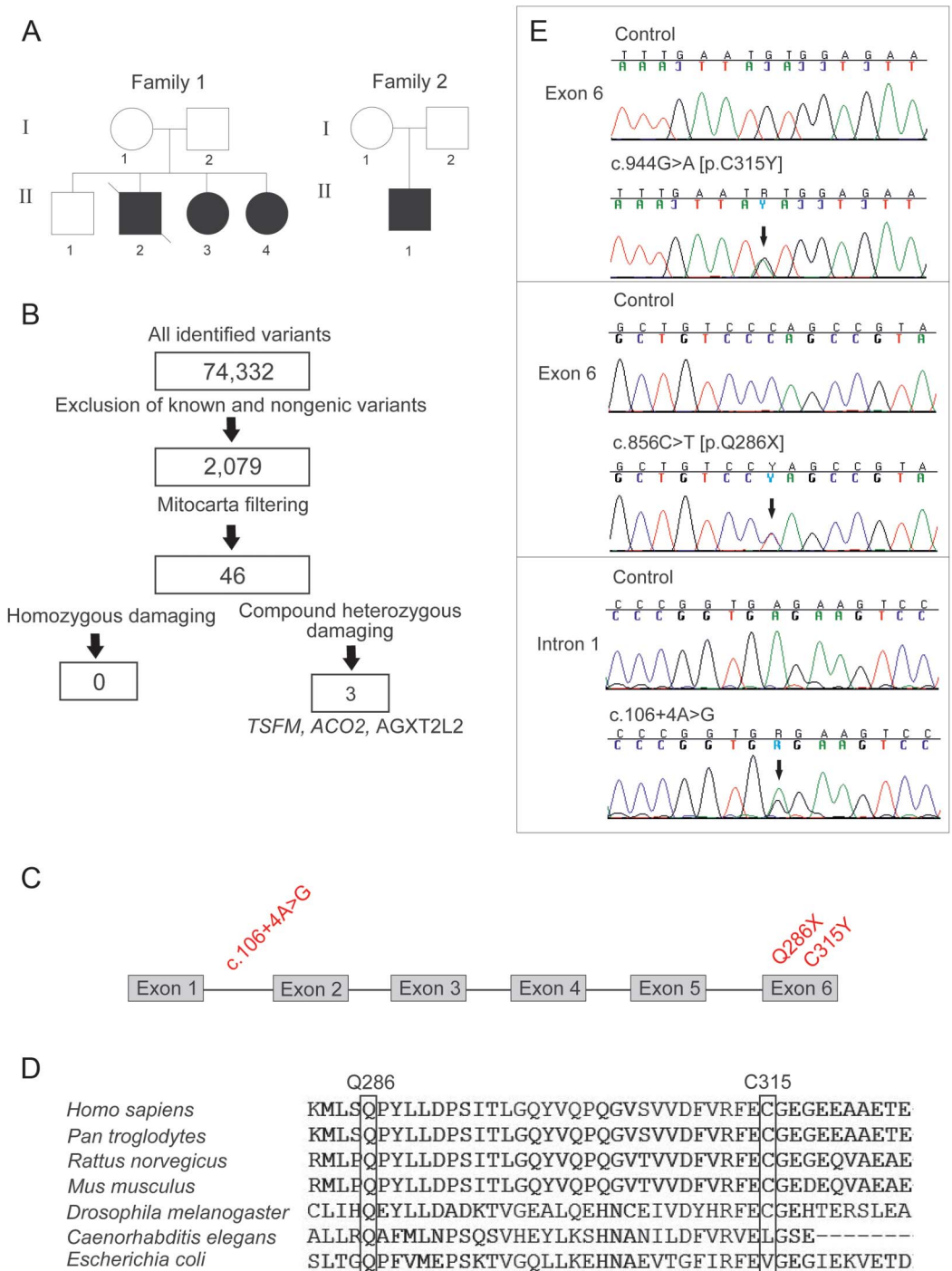
(A) The pedigrees. Black symbols indicate affected individuals; strike through indicates deceased subject. (B) Exome data analysis and filtering steps of the single nucleotide variants of patient 1 (P1, family 1, II-3). *TSFM, AGXT2L2* (*PHYKPL,* 5-phosphohydroxy-ʟ-lysine phospho-lyase) and *ACO2* (aconitase 2) indicate the genes in which potentially pathogenic variants were identified. *TSFM* gave best variant calling quality and was thus selected for further analysis. (C) *TSFM* gene structure with all 3 identified mutations marked in red. (D) Conservation of mutant elongation factor Ts amino acids Q286 and C315 in species. (E) Chromatograms of the 3 identified mutations (arrows). All mutations were confirmed to be heterozygous.

reads mapped to the reference sequence with 20-fold coverage for 49.8% of the target bases. Figure 1B summarizes the filtering steps. P1 was found to carry compound heterozygous mutations in the *TSFM* gene that have been previously linked to mitochondrial cardiomyopathies: exon 6, missense mutation c.944G>A, Genbank cDNA NM_005726 [p.C315Y] and nonsense mutation c.856C>T [p.Q286X] (figure 1C). p.C315 is highly conserved in species, down to *Drosophila melanogaster* (figure 1D). The p.Q286X deletes the C-terminal 39 amino acids, leading to a truncated protein. Sanger sequencing

confirmed the findings in P1 and in her affected sister P2 (figure 1E), and their parents were heterozygous carriers, indicating that the mutations cosegregated with the disease in the family. Sequencing the cDNAs from P1 and P2 fibroblast revealed that both alleles were present in cells, indicating that *TSFM* p.Q286X mRNA is not immediately degraded by nonsense-mediated decay.

A search for other *TSFM* variants in our mitochondrial disease patient cohort of 76 subjects revealed patient 3 (P3) to carry p.Q286X and a heterozygous intronic change c.106+4A>G. Sanger sequencing confirmed the findings. The mother of P3 carried the c.106+4A>G (figure 1E). This variant changed a conserved nucleotide within a splice site donor region, directly after the first exon (figure 1C), predicting aberrant splicing and a premature stop codon after 165 nucleotides. However, in cultured myoblasts of P3, c.106+4A>G did not affect *TSFM* transcript size, and when the alleles were quantified by solid-phase minisequencing, both were equally present in proportion. No other material was available from P3, and therefore we could not address a potential tissue-specific splicing defect. The total amount of *TSFM* transcript was reduced to 50% in P3 myoblasts, when compared with control myoblast lines (figure 2A), suggesting that the intronic mutation affected the general *TSFM* expression or the stability of the transcripts, possibly reducing the amount of *TSFM* available for translation.

Enrichment of p.Q286X mutation in the Finnish population. *TSFM* p.Q286X variant was identified as a potentially pathogenic change with an independent, bioinformatics strategy. Exome data of more than 3,000 Finns (SISu) were compared with similar data from other populations to find which mutations were present in the Finnish population with high heterozygous carrier frequency, but lacking homozygotes. The p.Q286X was found to be enriched in Finland, with a heterozygous carrier frequency of 1:90. This frequency was verified by separate genotyping of a total of 32,497 Finnish subjects indicating even more common carrier frequency, 1:80. Based on Hardy-Weinberg equilibrium, 1 to 2 homozygotes would be expected in this population, but none existed. Such a carrier frequency predicted that, of 60,000 children born in Finland every year, 2 to 3 should be homozygous for this *TSFM* mutation. However, such patients were not identified in our mitochondrial patient cohort, suggesting that homozygosity is lethal, potentially leading to prenatal death. We therefore sequenced this *TSFM* mutation site from a sample collection from 32 intrauterine fetal deaths. However, all of the fetuses screened were carrying the wild-type allele.

No carriers for p.C315Y and c.106+4A>G variants were found among the 3,000 subjects from the Finnish population (SISu). We also searched this database for other *TSFM* variants, which would change amino acids, and found 2 such variants with maximal frequency of 1:1,500 (table e-1 on the *Neurology*® Web site at Neurology.org). These results show that variants in EFTs are rare, with the exception of the allele carrying the p.Q286X change.

TSFM **mutations result in an unstable EFTs protein and reduced mitochondrial translation.** *TSFM* encodes for mitochondrial EFTs, which together with EFTu is essential for translation of mitochondrial DNA–encoded proteins. EFTu is a G protein, assisting in binding of charged aminoacyl-tRNA to the A-site of the mitochondrial ribosome in a guanosine triphosphate–dependent manner, and the EFTs functions as a nucleotide exchange factor. We used the previously resolved structure of the bovine EFTu-EFTs[31] complex to model the human proteins (figure 2B). Human and bovine EFTs sequences show 83.8% identity and 88.2% similarity, while EFTu sequences show 94.5% and 96.5%, respectively. Our modeling indicated that C315 locates in the EFTs subdomain C of the core domain and was predicted to have an entirely structural role, as explained in detail in figure 2B. Change in local architecture would affect stability of the protein because of a less densely packed core and also would affect the positions of both p.D126 and p.F127, shown to be essential for interaction within the Tu-Ts complex.[31] Therefore, p.C315Y change is likely to result in a largely unstable inactive protein and together with p.Q286X mutation to severely decreased amounts of EFTs.

As predicted by our structural analysis, EFTs was almost completely absent in P1 and P2 fibroblast mitochondria, and showed substantial reduction also in P3 myoblasts, in Western blot analysis, supporting the role of EFTs in the disease pathogenesis of all 3 patients (figure 2C). EFTu levels are known to follow EFTs levels,[2,33] and consistent with this, the EFTu amount was clearly reduced in P1 and P2 fibroblasts, but not in P3 myoblasts, compared with controls (figure 2C). We then performed pulse labeling of mitochondrial translation products on all 3 cell lines and detected a partial translation defect in P2 fibroblasts, a suggestive defect in P1, and a clear reduction of overall mitochondrial translation in P3 myoblasts, especially in the cytochrome *c* oxidase subunits (figure 2D).

DISCUSSION Herein, we report that *TSFM* mutations, leading to defects of mitochondrial translation elongation factor EFTs, can underlie childhood or juvenile-onset nervous system phenotypes, with or without cardiomyopathy. The infantile cardiac phenotype was severe and progressive, but later stabilized, whereas the nervous system phenotypes,

Figure 2 *TSFM* mutations lead to protein degradation and mild reduction in translation activity
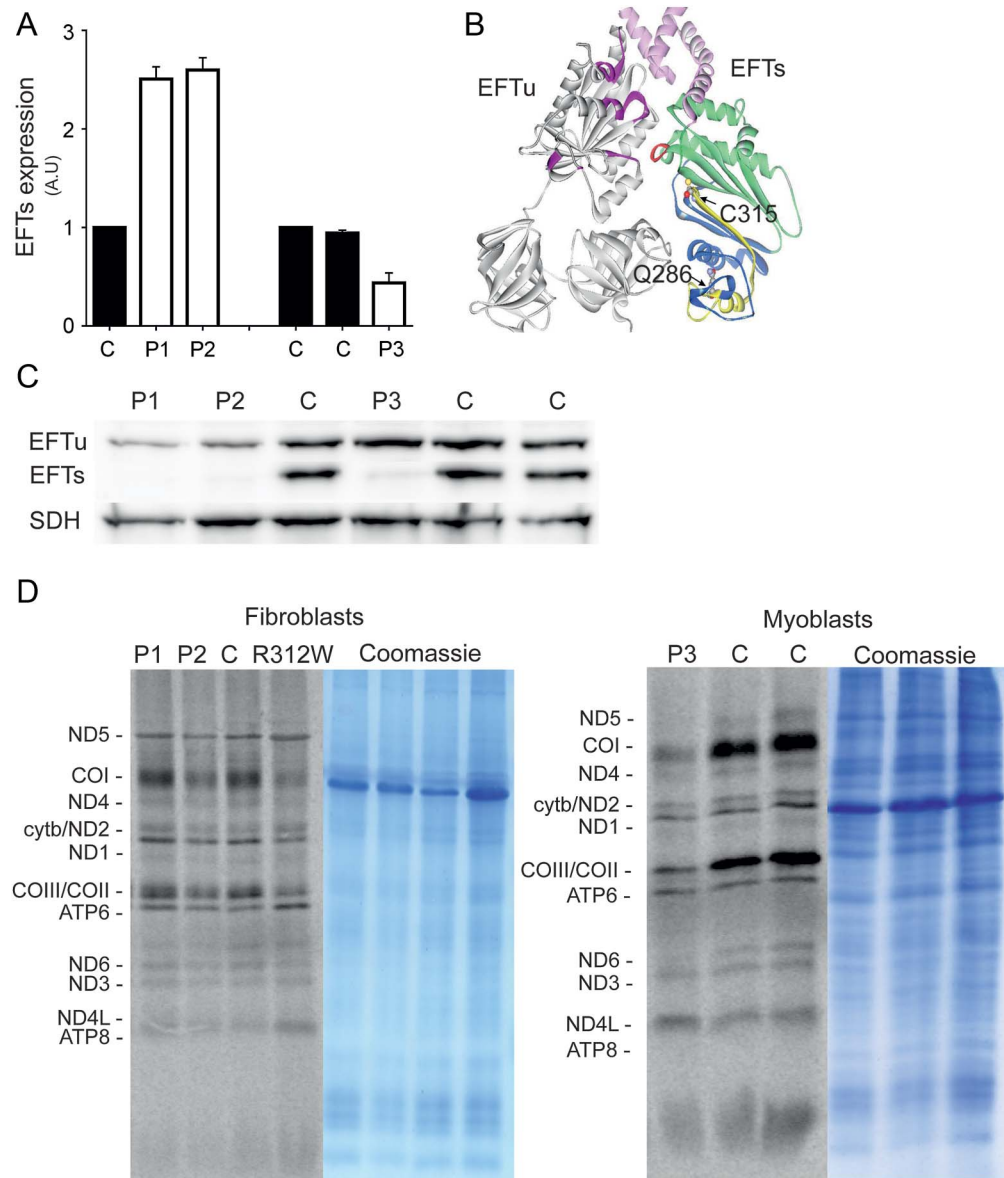
(A) Relative messenger RNA expressions of EFTs in cultured cells of P1, P2, and P3. (B) Structural modeling of EFTu-EFTs complex showing the 2 amino acid changes p.Q286X (deleted sequence in yellow) and p.C315Y. Color coding: gray = EFTu; magenta = residues forming the GDP binding site in EFTu; pink = EFTs amino terminal domain (residues 1–99); green = the core domain (residues 100–210); blue = subdomain C (residues 211–318); and red = EFTs interaction site with EFTu and nucleotide exchange. p.C315 fits tightly into an uncharged pocket predicted to be formed by the residues from the opposite side of the β-sandwich. Substitution of cysteine 315 for tyrosine introduces a bulky side chain into the pocket designed for cysteine, resulting in van der Waals repulsion between the halves of the β-sandwich oriented toward the EFTu contact surface. This change in the local architecture affects stability of EFTs. Structural rearrangements caused by the mutation will affect the position of p. E104 locating in a distance of 3.8 Å from p.D126, which forms part of the α-turn in the subdomain N (marked in red) critical for interaction with EFTu and nucleotide exchange. (C) Immunoblot analysis showed almost complete loss of EFTs protein in P1 and P2 fibroblasts and substantial reduction in P3 myoblasts. Mild EFTu reduction was also seen in P1 and P2 but not in P3 samples. (D) Mitochondrial translation was partially reduced in P2 fibroblasts, but suggestively P1. An evident reduction of translation was seen in P3 myoblasts. R312W = patient fibroblast cell line with previously published EFTs mutation as a positive control for translation defect. ATP = ATP synthase; CO = cytochrome c oxidase; cytb = cytochrome b; EFTs = elongation factor Ts; EFTu = elongation factor Tu; GDP = guanosine diphosphate; ND = NADH dehydrogenase; SDH = succinate dehydrogenase.

including optic atrophy, peripheral axonal neuropathy, Leigh disease, and/or ataxia, manifested in school age. Previously, *TSFM* mutations have been described to always be lethal in infancy, with cardiomyopathy, encephalomyopathy, or both,[3,5] or liver failure,[34] and all the reported patients carried the same homozygous change, p.R312W. Our results support the previous findings that mitochondrial translation defects manifest in early infancy as cardiomyopathy.[11,18,35] However, stabilization of the

heart condition suggests that the translation defect may be compensated, if the patient survived the early stage. Indeed, similar early-onset severe disorders, followed by stabilization of cardiomyopathy, have been previously reported in single patients with mitochondrial translation defects. MRPL44[11] and ELAC2[24] mutations cause mitochondrial cardiomyopathies with infantile lethality, but in the few surviving patients, cardiomyopathy transforms to slowly progressive in childhood. The lack of cardiomyopathy in patient 3 suggested that the residual EFTs was enough to rescue his heart, but not the nervous system, as he developed optic atrophy, neuropathy, and ataxia in juvenile age, mimicking the neurologic symptoms of the other 2 patients. Accumulation of data from the natural history of diseases, and their correlation with genotype information, may aid in treatment decisions, e.g., prioritization for heart transplantation.

We had the privilege of accessing an excessive genetic data collection of SISu. These data revealed that the p.Q286X mutation is highly enriched in the Finnish population, with 1:80 carrier frequency, but no homozygotes were found, strongly supporting a pathogenic role of this mutation. Very low frequency of other *TSFM* mutations in the population explained why defects in this gene are not causing a large proportion of mitochondrial diseases in Finland. Furthermore, the absence of p.Q286X allele homozygotes in mitochondrial disease, intrauterine death, and population cohorts suggested early developmental lethality. The frequency of the allele suggests it to potentially explain 2 to 3 early miscarriages per year in the country. These data emphasize the importance and power of large population-specific genomic data collections for identification of genetic causes for disease.

The pathogenic role of EFTs in all of our patients was strongly supported by the considerable reduction of EFTs protein in their samples, as well as consequent reduction in mitochondrial translation. This translation defect in fibroblasts was mild to moderate, despite very little residual EFTs, indicating that mitochondrial translation can be maintained with trace amounts of an essential factor. The overall reduction of *TSFM* transcript in P3, without an apparent splicing problem, leaves the pathogenic mechanism in his case still somewhat open. Cell-type–dependent splicing has been proposed to occur in nervous system manifesting disorders of mitochondrial translation, with increased probability to exon skipping especially in neural cell lines.[14,36] Whether such a tissue-type–specific sensitivity to splicing errors could contribute to EFTs consequences in our patients remains to be verified.

EFTs binds the EFTu–guanosine diphosphate (GDP) complex, mediates GDP release, and forms a stable EFTu-EFTs complex.[37,38] GDP can slowly dissociate from the protein, but in that transition state, EFTu becomes unstable[39] unless stabilized by bound EFTs. EFTs and Tu protein amounts closely follow each other, the levels reflected in the amount of mitochondrial translation products,[2,5,40] indicating the importance of their stoichiometric balance for mitochondrial translation. The β-sandwich structure of EFTs is important for interaction with Tu and stability of the complex.[39] Mutation p.C315Y in human Ts destabilizes the central part of the β-sandwich core, especially affecting the contact surface for interaction with Tu. This structural change occurs at a site adjacent to a motif essential for binding to Ts and the nucleotide exchange. This protein-modeling prediction is in line with our findings of reduction of EFTs levels, which was accompanied by reduction in EFTu levels and a slight decrease in translation.

Our findings show that EFTs disorders manifest with wide variability in phenotypes, extending from early-lethal multisystem disorders with cardiomyopathy to childhood-onset Leigh disease, optic atrophy, neuropathy, and juvenile-onset ataxia neuropathies. Our findings support the notion that EFTs and mitochondrial translation are especially critical for cardiac function in early childhood, and if the patient survived the critical years, the cardiac status may stabilize. This finding emphasizes aggressive treatment to support cardiac function at the early stage, because the patient may stabilize without a cardiac transplant. The p.Q286X mutation was found to be enriched in the Finnish population, however, without homozygotes even in the mitochondrial patient population. This finding indicates that this severe mutation can be causing intrauterine deaths, most likely due to early developmental cardiac dysfunction.

DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

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