Case Report

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Mutation in the mitochondrial translation elongation factor EFTs results in severe infantile liver failure

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Background & Aims: Multiple respiratory chain deficiencies represent a common cause of mitochondrial diseases and often result in hepatic failure. A significant fraction of patients present mitochondrial DNA depletion but a number of cases remain unexplained. The aim of our study was to identify the disease causing gene in a kindred with intrauterine growth retardation, neonatal lactic acidosis, liver dysfunction and multiple respiratory chain deficiency in muscle.

Methods: Homozygosity mapping was performed by 50K SNP genotyping and candidate genes were successively analyzed by direct sequencing on genomic DNA of the family members.

Results: SNP genotyping detected several regions of homozygosity in which we focused our attention to genes involved in mitochondrial translation. We sequenced the TSFM gene, encoding the mitochondrial translation factor EFTs and identified a homozygous mutation changing a highly conserved arginine into a tryptophan (R312W).

Conclusions: This mutation has been previously reported in two unrelated kindred presenting two distinct syndromes (fatal mitochondrial encephalomyopathy and hypertrophic cardiomyopathy respectively). The description of a third syndrome associated with a same TSFM mutation gives support to the broad clinical and genetic heterogeneity of mitochondrial translation deficiencies in human. It suggests that mitochondrial translation deficiency represents a growing cause of hepatic failure of mitochondrial origin in infants.

Introduction

Hepatic failure is a frequent feature in respiratory chain deficiency. Patients usually present with liver insufficiency, cholestasis, cirrhosis and/or cytolysis soon after birth or during the first weeks of life. Respiratory chain (RC) enzyme deficiency can be diagnosed on needle biopsy of the liver. The mitochondrial respiratory chain consists of five complexes composed of more than 80 different subunits, 13 of them being encoded by the mitochondrial genome (mitochondrial DNA, mtDNA). All complexes, except complex II, contain both nuclearly and mitochondrially encoded proteins. Mitochondrial genes are translated within the mitochondria by a specific translation apparatus comprising 2 ribosomal and 22 transfer RNAs (rRNA and tRNA) encoded by the mtDNA and hundreds of proteins (ribosomal proteins, amino-acyl tRNA synthetases, initiation, elongation, and termination factors) encoded by nuclear genes. Multiple RC enzyme deficiency with a severe decrease of liver mtDNA copy number (mtDNA depletion) has been found in a significant fraction of patients and ascribed to mutations in genes involved in mtDNA replication or dNTP supply for mtDNA replication (*POLG1* [1], *PEO1* [2], *DGUOK* [3], and *MPV17* [4]). Few cases of severe hepatic failure have been ascribed to genes in the mitochondrial translation machinery, namely *TRMU* [5], a mitochondrial tRNA-modifying enzyme and *GFM1* [6,7], a translation elongation factor. Yet a number of cases of liver failure and RC deficiency remain unexplained. Here, we show that the *TSFM* gene encoding EFTs, the mitochondrial translation elongation factor S, is a cause of severe liver failure. This supports the view that mitochondrial translation deficiency represents an increasing cause of early onset hepatic failure.

Patients and methods

Patient 1 (V-4), a girl, was the fourth child of consanguineous parents from Mali (Fig. 1). She presented intrauterine growth retardation and died at day 1 for unknown reasons. Patient 2 (V-6) was the sixth child of the sibship. She had intrauterine growth retardation and was born at 37 weeks of pregnancy. Birth weight was 2330 g, length 46 cm and head circumference 32 cm. Neurological examination was normal at birth but she refused feeding and was found to have persistent hyperlactatemia (lactate: 20 mmol/L, control values <2.2 mmol/L), anemia, and jaundice (total bilirubin: 17 μmol/L, control values <60 μmol/L). At 24 h of life, she developed slight trunk hypotonia and cytolysis (AST: 141 UI/L, control values: 10–40). Her condition worsened at 6 weeks of age. She had major trunk hypotonia, tubulopathy, and liver insufficiency. She died at 2 months of age. Patient 3 (V-7), the younger sister of the sibship was born at 39 weeks of pregnancy (birth weight 2350 g, length 47 cm, head circumference 34 cm). She had major trunk hypotonia, hypertrophic cardiomyopathy, poor spontaneous movements, and no sucking at birth. At day 2, she had persistent hyperlactatemia (lactate 4.4 mmol/L), cytolysis and cholestasis (AST: 47 UI/L, control values: 5–30; γGT: 36 UI/L, control values: 5–20; bilirubin 161 μmol/L, control values <60 μmol/L). Her neurological condition worsened from day 10, she had dystonic movements, generalized hypotonia, and she died at 2 weeks of age. Finally, the first girl of the sibship (V-1) was found to have an unrelated condition, minicore myopathy with normal RC enzyme in muscle.

Keywords: Mitochondria; Liver failure; Respiratory chain; Mitochondrial translation; TSFM.

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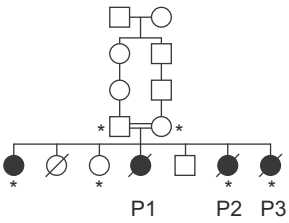

Fig. 1. Pedigree of the family. Black circles indicate patients with liver failure and RC deficiency. The first child has minicore myopathy and normal RC. Asterisks indicate individuals that have been SNP genotyped.

Respiratory chain analysis, protein analysis, antibodies and ECL detection

Spectrophotometric assays of respiratory chain enzymes were carried out as previously described [8]. For blue native–polyacrylamide gel electrophoresis (BN–PAGE), mitochondria and OXPHOS complexes were isolated as described [9]. Solubilized OXPHOS proteins (15 μg) were loaded on a 4–16% acrylamide non-denaturing gradient gel (Invitrogen). Immunodetection was performed using the following primary antibodies: anti-CI-Grim19, CII-SDHA, CIII-core2, CIV-COX1 and CV-subunit β (mouse monoclonal antibodies, Mitosciences). Secondary antibody detection was performed using peroxidase-conjugated anti-mouse IgGs (Abcam). The signal was generated using ECL (Pierce, Rockford, USA).

Homozygosity mapping and mutation screening

We performed SNP genotyping using the GeneChip Human Mapping 50K Array Xba 142 2.0 for individuals designed with an asterisk (Fig. 1). The exons of the *TSFM* gene were amplified using specific primers (Table 1) after an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a last extension at 72 °C for 10 min. Amplification products were purified by ExoSapIT (Amersham, Buckinghamshire, United Kingdom) and directly sequenced using the PRISM Ready Reaction Sequencing Kit (Perkin-Elmer, Oak Brook, IL) on an automatic sequencer (ABI 3130xl; PE Applied Biosystems, Foster City, CA).

Results

Respiratory chain enzyme and mtDNA analysis

Patients 2 and 3 presented reduced absolute activities of complexes I and IV in muscles as well as abnormal activity ratios. Cultured skin fibroblasts of patient 3 showed normal RC enzyme activities (Table 2). Large scale mtDNA deletion, mtDNA depletion and common point mutations (m.3243A>G, m.8344A>G, m.8993T>G) were excluded by using appropriate techniques in muscle DNA of patients 2 and 3.

Table 2. Respiratory chain activities in muscle mitochondria and fibroblasts.

	Muscle				Fibroblasts	
	homogenate		mitochondria			
	P2	C	P3	C	P3	C
Absolute activities (nmol/min/mg prot)						
CI	**3**	8-32	**10**	47-182		
CII	16	18-70	110	69-268	14	11-42
CIII	64	67-268	587	421-1654	112	50-187
CIV	**16**	86-342	**235**	607-2419	84	50-197
CV			242	220-862		
CI+III			**41**	138-549		
CII+III	18	17-63	206	171-679	39	18-68
CS	59	54-210	107	54-210	94	37-143
Activity ratios						
CIV/CI	**5.7**	10.6 ± 0.8	23.5	10.9 ± 2.0		
CIV/CII	**1.0**	5.4 ± 0.4	2.1	7.8 ± 0.7	6.0	4.8 ± 0.4
CIV/CIII	**0.2**	1.3 ± 0.1	**0.4**	1.2 ± 0.1	0.8	1.0 ± 0.1
CIV/CV			**1.0**	2.6 ± 0.2		
CIV/CI+III			**5.7**	3.7 ± 0.5		
CIV/CII+III	**0.9**	5.4 ± 0.6	**1.1**	3.3 ± 0.4	2.1	2.9 ± 0.2

CI–CV, complexes I–V; CS, citrate synthase; LM, lauryl-maltoside; P1–P4, patients 1–4; C, control; abnormal values are in bold.

BN–PAGE analysis

BN–PAGE analysis of mitoplasts isolated from skin fibroblasts of patient 3 revealed a marked decrease of fully-assembled complexes I and IV as compared to controls whereas complex II was normal (Fig. 2). Moreover, lower molecular weight subcomplexes were observed following incubation with anti-GRIM-19, and anti-ATPase β antibodies. This result was consistent with a combined RC deficiency affecting mtDNA encoded complexes and possibly a translation deficiency.

Homozygosity mapping and TSFM sequencing

SNP genotyping using 50K Xba array allowed to identify one region of homozygosity of 40 Mb on chromosome 12 (chr12:29,624,783-69,537,647). This region encompassed more than 440 genes, 19 of them encoding either mitochondrial proteins or proteins predicted to be targeted to the mitochondria. Moreover, two large regions of 12 and 30 Mb on chromosomes 14 (chr14:80,074,862-92,786,599) and 19 (chr19:9,660,405-49,024,587) could not be excluded. As the abnormal BN–PAGE profile observed in patient 3 was suggestive of mitochondrial translation deficiency, we focused our interest on genes encoding

Table 1. Oligonucleotides for TSFM sequence analysis.

Exons	Primer sequence	
	Forward	Reverse
1-2	AGCAGGTGGCACCATACTC	TGCCATTATGGTCACTGAAG
3	TGGTAGACTGCCAGTAAATGATAG	TCTTCAAGAACAGGCCCC
4	TTTCCGTTGAGTCTGTAGCTTG	AGCATTTAAGCAGAGATGTGAAG
5	ACGCCTGGCCAATACTTTTC	CACACCACTTCTCACGTTCG
6	GACTGTCTTCCAAACTGGGC	CTCGGTCTGAAGAGGTTTGG

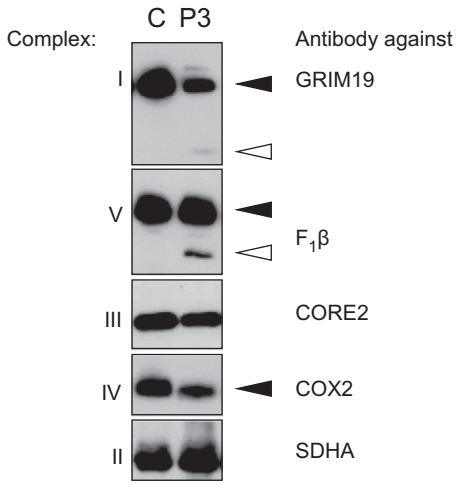

Fig. 2. BN–PAGE analysis of mitochondria from cultured skin fibroblasts of patient 3 (P3) and control (C) using antibodies against GRIM19 for complex I (CI), SDHA for complex II (CII), core 2 for complex III (CIII), COX2 for complex IV (CIV), and $F_1\beta$ subunit for complex V (CV). Filled arrows indicate low amount of CI, CIV, and CV. Empty arrows indicate additional bands.

proteins of the mitochondrial translation machinery namely *YARS2* and *TSFM* on chromosome 12, *MRPL4*, *MRPL34*, *MRPS12*, *GTPBP3* and *SARS2* on chromosome 19. After having excluded mutations in the coding sequences and exon–intron boundaries of *YARS2*, *MRPL4*, *MRPL34*, *GTPBP3* and *SARS2*, we identified a homozygous C to T transversion at nucleotide 934 in exon 6 of the *TSFM* gene in patients 3–4. This mutations changed a highly conserved arginine into a tryptophan (R312W, relative to Genbank accession number NM_005726, corresponding to R333W previously reported). The two parents, individual V-1 and the healthy sister (V-3) were heterozygous for this mutation.

Discussion

Here, we report on a homozygous *TSFM* mutation in a case of neonatal onset hepatic failure and multiple RC deficiency. This *TSFM* mutation was identified in a consanguineous family by combining SNP genotyping and candidate gene approach based on an abnormal BN–PAGE profile. The deleterious nature of the R312W mutation has been previously established by expression studies in patient's fibroblasts demonstrating that the mutation is indeed the cause of the disease and results in an abnormal translation of mitochondrially-encoded proteins [10].

TSFM mutations have been rarely reported and therefore represent a rare cause of mitochondrial disorders. Previously reported patients had fatal mitochondrial encephalomyopathy, hypertrophic cardiomyopathy [10] or both [11]. Hitherto however, *TSFM* mutations had never been reported as a cause of hepatic failure in human. The clinical heterogeneity of *TSFM* mutations is particularly intriguing as all patients presented exactly the same mutation. Intrafamilial heterogeneity also occurs here as patient 3, but not patient 2, had hypertrophic cardiomyopathy at day 2. Unknown factors possibly related to specific polymorphisms in other translation factors, genetic background, adaptive mechanisms and/or susceptibility to external factors should contribute to this clinical heterogeneity.

Liver failure is a common feature in multiple RC deficiencies. Some patients present severe liver mtDNA depletion related to *DGUOK* [3] *POLG1* [1], *PEO1* [2] or *MPV17* [4] mutations. Others had translation deficiency caused by *TRMU* and *GFM1* mutations [5–7]. The observation of *TSFM* mutations in two patients with liver failure suggests that *TSFM* should now be regarded as a disease gene in hepatic failure. Mitochondrial translation deficiency represents therefore a cause of hepatic failure in neonates. Yet it should be borne in mind that a mutation in genes of the translation machinery was a rare cause of liver failure in our series as 3/74 patients with liver dysfunction were found to carry *GFM1* (one patient) and *TRMU* mutations (two patients).

The family reported in this study is part of a cohort of 101 patients with isolated hepatic failure and combined RC deficiency. Among them, 26.7% present severe mtDNA depletion related to mutations in *DGUOK*, *POLG1*, *MPV17* or *PEO1*. Whether the remaining 70 patients with normal mtDNA content and no *TRMU*, *GFM1* and *TSFM* mutations present translation deficiency has still to be determined. Nevertheless, the systematic study of mtDNA allowed to exclude mtDNA mutations in all patients. Therefore, this emphasizes that nuclear gene mutations underlie these mitochondrial hepatic failures.

In conclusion, while mtDNA depletion has long been regarded as a frequent cause of hepatocellular insufficiency or liver failure (*DGUOK* [3], *POLG1* [1], *MPV17* [4] and *PEO1* [2] mutations), we suggest to consider abnormal mitochondrial translation as a novel cause of liver failure in the respiratory chain deficiency.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Ferrari G, Lamantea E, Donati A, Filosto M, Briem E, Carrara F, et al. Infantile hepatocerebral syndromes associated with mutations in the mitochondrial DNA polymerase-gammaA. Brain 2005;128:723–731.
[2] Sarzi E, Goffart S, Serre V, Chretien D, Slama A, Munnich A, et al. Twinkle helicase (PEO1) gene mutation causes mitochondrial DNA depletion. Ann Neurol 2007;62:579–587.
[3] Mandel H, Szargel R, Labay V, Elpeleg O, Saada A, Shalata A, et al. The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. Nat Genet 2001;29:337–341.
[4] Spinazzola A, Viscomi C, Fernandez-Vizarra E, Carrara F, D'Adamo P, Calvo S, et al. MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. Nat Genet 2006;38:570–575.
[5] Zeharia A, Shaag A, Pappo O, Mager-Heckel AM, Saada A, Beinat M, et al. Acute infantile liver failure due to mutations in the TRMU gene. Am J Hum Genet 2009;85:401–407.
[6] Coenen MJ, Antonicka H, Ugalde C, Sasarman F, Rossi R, Heister JG, et al. Mutant mitochondrial elongation factor G1 and combined oxidative phosphorylation deficiency. N Engl J Med 2004;351:2080–2086.
[7] Antonicka H, Sasarman F, Kennaway NG, Shoubridge EA. The molecular basis for tissue specificity of the oxidative phosphorylation deficiencies in patients with mutations in the mitochondrial translation factor EFG1. Hum Mol Genet 2006;15:1835–1846.
[8] Rustin P, Chretien D, Bourgeron T, Gerard B, Rotig A, Saudubray JM, et al. Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta 1994;228:35–51.
[9] Nijtmans LG, Henderson NS, Holt IJ. Blue Native electrophoresis to study mitochondrial and other protein complexes. Methods 2002;26:327–334.

[10] Smeitink JA, Elpeleg O, Antonicka H, Diepstra H, Saada A, Smits P, et al. Distinct clinical phenotypes associated with a mutation in the mitochondrial translation elongation factor EFTs. Am J Hum Genet 2006;79: 869–877.

[11] Smits P, Antonicka H, van Hasselt PM, Weraarpachai W, Haller W, Schreurs M, et al. Mutation in subdomain G' of mitochondrial elongation factor G1 is associated with combined OXPHOS deficiency in fibroblasts but not in muscle. Eur J Hum Genet 2011;19:275–279.