ORIGINAL ARTICLE

Mitochondrial Infantile Liver Disease due to *TRMU* Gene Mutations: Three New Cases

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Abstract Combined respiratory chain defect is a common feature in mitochondrial liver disease during early infancy. Mitochondrial DNA depletions, induced by mutations of the nuclear genes *POLG*, *DGUOK*, and *MPV17*, are the major causes of these combined deficiencies. More recently, mutations in *TRMU* gene encoding the mitochondrial tRNA-specific 2-thiouridylase were found in infantile hepatopathy related to mitochondrial translation defect. It is characterized by a combined defect of respiratory chain complexes without mitochondrial DNA depletion.

We report here clinical, biochemical, and genetic findings from three unrelated children presenting with hepatopathy associated with hyperlactatemia and respiratory chain defect due to bi-allelic mutations in *TRMU* gene. Two patients recovered spontaneously in a few months, whereas the other one died of acute liver failure. Spontaneous remission is a rare feature in mitochondrial liver diseases, and early identification of *TRMU* mutations could impact on clinical management. Our results extend the small number of *TRMU* mutations reported in mitochondrial liver disorders and allowed accumulating data for genotype–phenotype correlation.

Introduction

Liver involvement is a frequent clinical presentation of neonatal mitochondrial cytopathies (García-Cazorla et al. 2005). In most cases, liver biochemical analysis showed a combined deficiency of mtDNA-dependent complexes (I, III, IV, and V) with a reduction of mtDNA copy numbers (depletion), due to mutations in nuclear genes encoding proteins implicated in mtDNA stability, mainly *POLG*, *DGUOK*, and *MPV17* genes (Naviaux and Nguyen 2004; Mandel et al. 2001; Spinazzola et al. 2006; Sarzi et al. 2007). In a few cases, activities of mtDNA-dependent complexes in liver are decreased, whereas mtDNA copy number is normal: a defect in mitochondrial protein synthesis machinery is then to consider. Mutations of three nuclear gene-encoding proteins involved in mitochondrial protein translation have been recently associated with liver disorders in the neonatal period: *GFM1*, *TUFM*, and *TRMU* (Coenen et al. 2004; Valente et al. 2007; Zeharia et al. 2009).

First, *TRMU* mutations in infantile mitochondrial hepatopathy were reported by Zeharia et al. in 2009 by homozygosity mapping in a cohort of 13 patients of predominately Yemenite Jewish origin (OMIM 610230) (Zeharia et al. 2009). Nuclear *TRMU* gene encodes the mitochondrial tRNA-specific 2-thiouridylase 1 (EC 2.1.1.61), responsible for the 2-thiolation of uridine at the first anticodon position of the mitochondrial tRNALys, tRNAGlu, and tRNAGln (Hagervall et al. 1987). This post-transcriptional modification of uridine in the wobble position contributes to precise and

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efficient codon recognition (Umeda et al. 2005). Defect of TRMU protein leads to a reduced 2-thiolation and a decrease of mitochondrial tRNA level that may impair the translation of mtDNA-dependent complexes and cause combined RC defect in liver (Zeharia et al. 2009; Guan et al. 2006).

Since 2009, to the best of our knowledge, only two other cases of TRMU mutations associated with neonatal hepatopathy have been described (Schara et al. 2011; Uusimaa et al. 2011). In this study, we report clinical, biochemical, and genetic findings in three unrelated patients affected by cholestasis, lactic acidosis, recurrent hypoglycemia, and combined respiratory chain (RC) deficiency. All harbored pathogenic TRMU mutations, including two novel mutations.

Material and Methods

Patients

We studied three unrelated patients with combined hepatic mitochondrial RC defect. Clinically relevant results are summarized next.

Patient 1 (P1) was born at term after an uneventful pregnancy and delivery (Apgar score 9/10) with a height of 47 cm, a body weight of 2.830 kg, and a head circumference of 33 cm. Her mother was Caucasian and her father was Asian. At 3 months of age, she presented with faltering growth, vomiting, and poor feeding. At 6 months, clinical examination confirmed growth retardation: height 58 cm (−3 SD), weight 4.970 kg (−3 SD), head circumference 38 cm (−3 SD). Hepatomegaly (without splenomegaly) and jaundice were also noticed. Abdominal ultrasound disclosed hyperechogenic liver, without ascites. The child presented axial and peripheral hypotonia. Cerebral MRI (magnetic resonance imaging) revealed myelination delay, without abnormal lactate peak on spectroscopy examination.

Laboratory investigations showed elevated serum transaminase activities when compared to upper limit of normal (ULN) (AST 8ULN, ALT 4ULN), cholestasis (total bilirubin 84 μmol/L, N < 17 μmol/L, conjugated bilirubin 71 μmol/L, N < 10 μmol/L) with elevated gamma-glutamyltransferases (GGT 6ULN). Factor V was normal (85 %). CPK and alpha-fetoprotein were 55 UI/l (N < 240 UI/L) and 28780 UI/ml (N < 25 UI/mL), respectively. Metabolic analysis revealed recurrent hypoglycemia and hyperlactatemia (5.0 mmol/L, N < 1.8 mmol/L) with elevated lactate/pyruvate ratios (L/P 28, N = 7–15). Lactate concentration in cerebrospinal fluid (CSF) was also increased (3.3 mmol/L, N < 1.9 mmol/L) with a slightly elevated L/P ratio (17, N = 7–15).

Liver histology showed a micronodular cirrhosis, an important canalicular cholestasis, and some oncotic change in hepatocytes. Microvesicular steatosis was absent.

Within a few months, her condition worsened and she was considered for a liver transplantation. Unfortunately, she died from variceal bleeding due to portal hypertension at 8 months of age.

Patient 2 (P2) was born at term from healthy unrelated Caucasian parents after an uneventful pregnancy and delivery (Apgar score 10/10, height 50 cm, weight 3.455 kg, head circumference 35 cm). At 4 months of age, hepatomegaly (without splenomegaly) and jaundice were detected. Abdominal ultrasound revealed hyperechogenic enlarged liver, with diffuse steatosis. Clinical examination noted regular growth and normal axial and peripheral tone. Cerebral MRI showed normal myelination but abnormal lactate peak on spectroscopy. Routine biochemical analysis disclosed similar liver disorders as P1 (AST 17ULN, ALT 13ULN, total bilirubin 42 μmol/L, conjugated bilirubin 21 μmol/L, GGT 11ULN) without coagulopathy (factor V 85 %). CPK and alpha-fetoprotein were 42 UI/L and 100600 UI/mL, respectively. P2 presented recurrent hypoglycemia and hyperlactatemia (8.0–10.0 mmol/L) with elevated lactate/pyruvate ratios (L/P 42–53). In CSF, lactate concentration was also increased (3.9 mmol/L) with a slightly elevated L/P ratio (18). Liver histology showed portal and perisinusoidal fibrosis with microvesicular steatosis and oncocytic hepatocytes.

P2 is currently 2 years old. Tailored diet allows good tolerance to fasting. Physical examination shows persistent liver enlargement, and abdominal ultrasound discloses some liver nodules (<1 cm).

Patient 3 (P3) is the third child of consanguineous Egyptian parents. At 33 weeks of gestation, intrauterine growth retardation was detected and the delivery was triggered 1 week later because of hemorrhagic placenta previa (delivery 36 weeks + 1 day, Apgar score 2/7/10, height 46 cm, weight 1.765 kg, head circumference 31 cm).

At 2 days of age, P3 presented cholestatic jaundice with hepatomegaly, hepatocellular deficiency (prothrombin time 45 %), and hypoglycemia with favorable outcome.

At 4 months of age, clinical examination noted hepatomegaly with splenomegaly. Abdominal ultrasound revealed several small liver nodules. P3 also presented growth retardation (height 55 cm [−3 SD], weight 4.400 kg [−2.5 SD], head circumference 39 cm [−2 SD]) and slight axial hypotonia. Cerebral MRI was not performed.

Biochemical investigations disclosed raised aminotransferase serum activities (AST 5ULN, ALT 2ULN), elevated serum bilirubin concentration (total 84 μmol/L, conjugated 63 μmol/L) and alpha-fetoprotein concentration (208000 UI/mL) but normal prothrombin time and GGT. Lactate concentrations were increased in blood (4.0–6.0 mmol/L; L/P 30–35) and slightly elevated in CSF (2.0 mmol/L, L/P not determined).

Table 1 Mitochondrial enzymatic activities in liver biopsies. Abnormal results are indicated in bold

	Patient 1 (liver)	Patient 2 (liver)	Patient 3 (liver)	Normal range
Mitochondrial enzymatic activities (nmol.min^{-1}.mg^{-1} of proteins)				
Complex I	**7**	**7**	**7**	19–26
Complex II	188	311	256	168–277
Complex III	248	400	238	143–192
Complex IV	**193**	**157**	**123**	202–319
Complex V	229	597	325	74–167
Citrate synthase	153	315	233	63–131
Activities ratios				
Complex IV/complex I	**27.6**	**22.4**	**17.6**	6.0–10.0
Complex II/citrate synthase	1.2	1.0	1.1	0.8–2.4
Complex IV/citrate synthase	**1.3**	**0.5**	**0.5**	2.5–3.3

Liver biopsy showed patent signs of fibrosis, irregular cirrhosis with nodulation, severe cholestasis, and moderate macrovesicular steatosis. Some oncocytic and swollen hepatocytes were detected.

At 5 years of age, this patient is still alive. Blood tests, clinical liver and neurological examinations were normal, but abdominal ultrasound noted a persistent multinodular liver.

Biochemical Assays

Mitochondrial enzymatic activities were measured in liver biopsies from P1, P2, and P3, in muscle biopsy from P1, and in cultured fibroblasts from P1 and P2. Fibroblasts were cultured in HAM F10 medium with 10 % FCS. Enzymatic activities of RC complexes (I, II, III, IV, and V) and mitochondrial enzyme marker (citrate synthase) were performed according to Rustin et al. (1994).

Molecular Analysis

Mutations in mtDNA were screened by sequencing the whole mitochondrial genome, and mtDNA copy numbers were measured in liver by quantitative PCR based on the ratio of mtDNA to nuclear DNA (*MTND2/ATP5B*) (Chabi et al. 2003).

Exon and exon–intron junctions of *POLG*, *DGUOK*, *MPV17*, and *TRMU* genes were sequenced on genomic DNA (*TRMU* reference sequence NM_018006).

Putative mutations were validated by sequencing PCR products on both strands. The segregation of alleles was confirmed by parents' DNA analysis.

For the new point mutation (p.Glu217Lys), 100 alleles from healthy Caucasian subjects were sequenced on the exon 5. The tool PolyPhen-2 based on multiple-sequence alignment was used to assign a score from 0.00 (benign) to 1.00 (probably damaging) reflecting the impact of amino acid substitution (www.genetics.bwh.harvard.edu/pph2).

For P1, total RNA was extracted from cultured fibroblasts, and the *TRMU* cDNA was sequenced using standard procedure.

Written informed consent was obtained from parents of each patient.

Results

Enzymatic Activities

Results of the activities of RC complexes in liver are summarized in Table 1. Deficiencies of complexes I and IV were detected in liver biopsies of every patient. The activity ratio of complex IV to complex I was strongly increased, suggesting that the enzymatic defect was more pronounced for complex I than for complex IV.

Muscle biopsy from P1 was also carried out because of axial hypotonia. The activities of RC complexes were found markedly reduced: complex I 4 nmol.min^{-1}.mg^{-1} of proteins (normal range 16–52); complex II 12 nmol.min^{-1}.mg^{-1} of proteins (normal range 43–102); complex III 31 nmol.min^{-1}.mg^{-1} of proteins (normal range 125–418); complex IV 85 nmol.min^{-1}.mg^{-1} of proteins (normal range 125–520); complex V not determined. Normal citrate synthase activity (84 nmol.min^{-1}.mg^{-1} of proteins, normal range 69–240) indicated that the decrease of the activities of RC complexes was not caused by low mitochondrial content in the tissue.

In cultured fibroblasts from P1, the activities of complexes III and IV were decreased, whereas the activities of complexe II and citrate synthase were normal: complex II 18 nmol.min^{-1}.mg^{-1} of proteins (normal range 11–17); complex III 62 nmol.min^{-1}.mg^{-1} of proteins (normal range 98–180);
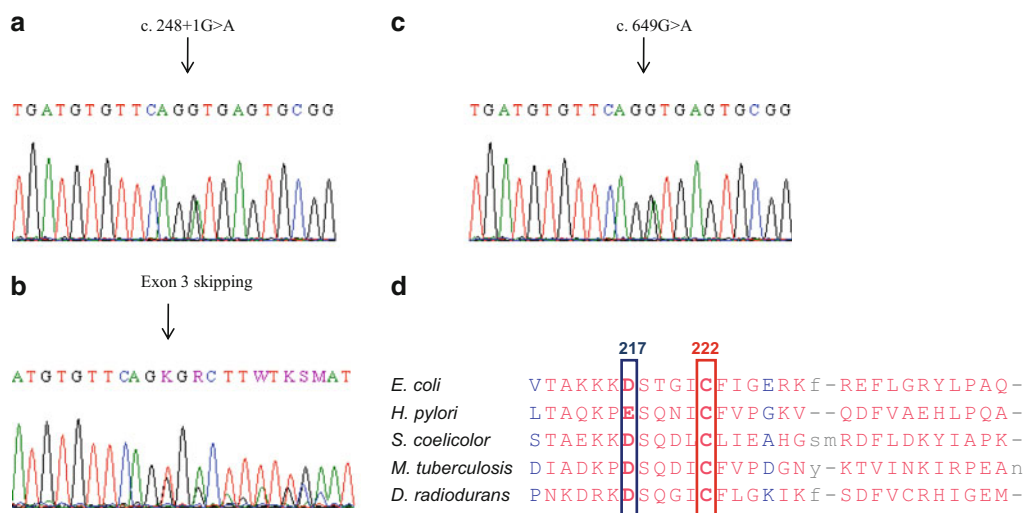
Fig. 1 Analysis of novel *TRMU* mutations. (**a**) Sequencing analysis of *TRMU* on P1 genomic DNA with the c. 248 + 1G>A heterozygous mutation. (**b**) Sequencing analysis of *TRMU* on P1 cDNA showing the exon 3 skipping. (**c**) Sequencing analysis of *TRMU* on P2 genomic DNA with the c. 649G>A heterozygous mutation (p.Glu217Lys). (**d**) Amino acid sequence of the conserved domain of the tRNA methyl-transferase family with the active Cys residue in position 222. Amino acids with carboxylic acid functional group (Glu or Asp) are conserved in position 217

complex IV 57 nmol.min^{-1}.mg^{-1} of proteins (normal range 72–143); complexes I and V not determined; citrate synthase 79 nmol.min^{-1}.mg^{-1} of proteins (normal range 32–72).

For P2, the activities of RC complexes were normal in cultured fibroblasts.

Genetic Analysis

A combined RC defect may result from defect of mtDNA maintenance, leading to mtDNA depletion, or alterations in mitochondrial proteins synthesis machinery. Liver mtDNA copy numbers were 40 %, 114 %, and 86 % of controls for P1, P2, and P3, respectively, excluding a mtDNA depletion. The sequencing of key nuclear genes involved in mtDNA instability in liver (*POLG*, *DGUOK*, and *MPV17*) did not show pathogenic mutations. Then, when sequence analysis of the entire mtDNA from liver failed to reveal mutations in either tRNA or rRNA genes, we considered a translation defect caused by mutations in a nuclear gene. *TRMU* gene emerged as a good candidate in the context of infantile hepatopathy. *TRMU* mutations were found in patients 1 and 2: c.835G>A/c.248 + 1G>A (P1); c.835G>A/c.649G>A (P2). Patient 3 was homozygous for the c.697C>T mutation. The c.835G>A (p.Val279Met) and c.697C>T (p.Leu233Phe) mutations have previously been described by Zeharia et al. (2009). We report here two new mutations: c.248 + 1G>A and c.649G>A (p Glu217Lys), both predicted to be highly damaging for protein function.

The new intronic c.248 + 1G>A mutation alters the natural 5' splice donor site of intron 2 (Fig. 1a). cDNA analysis on cultured fibroblasts from P1 showed that it leads to exon 3 skipping (Fig. 1b). The predicted resulting protein is likely to be inactive because it is truncated before the active site Cys222 that mediates uridine thiolation (p.Ser83ArgfsX18).

The missense c.649G>A mutation causes a p.Glu217Lys transition (Fig. 1c). A study of 100 control alleles excludes a common polymorphism. Evolutionary conservation of the Glu217 residue across orthologous genes is strong (conservation score Polyphen 0.999), indicating a structurally or functionally important role in the thiouridylase activity. Moreover, the Glu217 residue is situated in a conserved domain of the tRNA methyl-transferase family (pfam O3054), which includes TRMU protein, and is close to the Cys222 residue that mediates the thiolation of the tRNAs. As a consequence, the exchange between a carboxylic amino acid (Glu) and a basic amino acid (Lys) in position 217 is likely to be a disease-causing mutation (Fig. 1d).

Discussion

TRMU gene encodes a thiouridylase necessary to the maturation of mitochondrial tRNA and a correct translation (Umeda et al. 2005; Hagervall et al. 1987). The role of TRMU protein defect in mitochondrial diseases was first mentioned to modulate the phenotypic manifestation of the deafness-associated mitochondrial 12S rRNA mutation (m.1555A>G) (Guan et al. 2006; Yan et al. 2006). In 2009, Zeharia et al. first described *TRMU* mutations in mitochondrial hepatopathy characterized by infantile liver

injury with a decrease of mtDNA-encoded complexes in liver without mtDNA depletion (Zeharia et al. 2009). Only two other cases of *TRMU*-related hepatopathy have been described since (Kemp et al. 2011; Low et al. 2008; Schara et al. 2011; Uusimaa et al. 2011).

We report a series of three unrelated cases of infantile liver disease caused by *TRMU* mutations. All three children suffered from hepatomegaly with cytolysis and cholestasis in the first months of life. Two of them also presented growth retardation and abnormal tone. In all cases, hyperlactatemia with elevated lactate/pyruvate ratio suggested a RC defect.

The activities of RC complexes studies were undertaken in liver, muscle, and fibroblasts and our results clearly showed that liver biopsy is the most discriminating tissue to direct molecular investigations toward a RC combined defect. In all cases, strong decreases of complexes I and IV were noticed in the liver. The activities of complexes I and IV were diminished in all other cases of *TRMU*-related RC combined defects, even if other complexes may also be reduced (Zeharia et al. 2009; Low et al. 2008; Schara et al. 2011).

The activities of RC complexes could also be diminished in muscle biopsy, but not in all cases. Previous studies reported normal activities of complexes I, II, II + III, and IV (Zeharia et al. 2009) or deficiencies of complex IV (Zeharia et al. 2009), complexes I and IV (Schara et al. 2011; Low et al. 2008), or complexes I, II + III, and IV (Zeharia et al. 2009). In muscle biopsy from P1, mtDNA-encoded complexes I, III, and IV activities were decreased (V not determined), but, intriguingly, the nuclear-encoded complex II activity was also reduced. Decrease of complex II activity may reflect a secondary defect, since it has been suggested that TRMU protein deficiency could alter the addition of clusters Fe–S to complex II subunits (Sasarman et al. 2011).

Fibroblasts are the less informative tissue: a decrease of mtDNA-dependent complexes III and IV was noted in cultured fibroblasts from P1, whereas no abnormality was detected in fibroblasts from P2.

Following the identification of combined RC defect in liver, a depletion syndrome was first envisaged, but mtDNA depletion screening in liver and sequencing of the main genes involved in mtDNA maintenance failed to reveal anomalies. A translation defect was then investigated. As mitochondrial tRNA or rRNA mutations were excluded by whole sequencing of liver mtDNA, mutations in a nuclear gene were suspected. In addition to the *TRMU* gene, two genes have also been recently associated with early-onset liver disorders by translation defect: *GFM1* (elongation factor EFG1) and *TUFM* (elongation factor EFTu). Nevertheless, the *TRMU* gene emerged as the best candidate gene, because liver dysfunction was present and predominant in all cases described (Valente et al. 2007; Galmiche et al. 2012; Zeharia et al. 2009). Pathogenic *TRMU* mutations were found in every patient: c.835G>A (p.Val279Met)/c.248 + 1G>A (splicing alteration) (P1); c.835G>A/c.649G>A (p.Glu217Lys) (P2); c.697C>T (p.Leu233Phe)/c.697C>T (P3).

In spite of the similarity of the initial clinical and biochemical findings, the outcome of these patients was very different. P1 died at 6 months owing to liver failure, whereas P2 and P3 survived. At 2 and 5 years of age, respectively, they are developing normally. However, regular medical follow-up is maintained, particularly because of the persistence of liver nodules that could eventually be at risk of malignant transformation. The proportion of spontaneous clinical recovery after the acute episode in our series was similar to previously reported cases (11/15) (Zeharia et al. 2009; Uusimaa et al. 2011; Schara et al. 2011). Several hypotheses have been proposed to explain these spontaneous remissions, which are quite rare in clinical courses of mitochondrial cytopathies. Zeharia et al. suggested that a transient lack of the sulfur donor, cysteine, during the newborn period could aggravate the defect of tRNA thiouridylation and lead to mitochondrial dysfunction. Biochemical abnormalities could regress with the increase of cysteine availability during infancy, which explains the reversible phenotype (Zeharia et al. 2009). However, no experimental data support this theory. Another recent study claimed that a TRMU protein defect in fibroblast cell lines leads to a reduction of the 2-thiolation of mitochondrial tRNA but does not affect mitochondrial translation in normal conditions. Therefore, these authors proposed that the reduced level of modified mitochondrial tRNA could be a limiting factor only during early development (Sasarman et al. 2011).

We postulate that the hypothesis of a genotype and phenotype correlation could also be raised. Considering previous reported cases and our three new cases, it appears that among the five patients who died owing to *TRMU*-related hepatopathies, three carried one allele with a frameshift mutation or a splicing mutation resulting in a protein truncated before the active site Cys222: patient P1 reported here (p.Val279Met/p.Ser83ArgfsX18, c.248 + 1G>A) and two cases reported by Zeharia et al. (p.Tyr77His/p.Ser83-ArgfsX18, c.706-1G>A ; p.Val279Met/p.Ala167GlufsX36) (Zeharia et al. 2009). The other two patients who died were homozygous for a missense mutation at the first Met (p.Met1Lys/p.Met1Lys), that is predicted to be absolutely deleterious for protein activity (Zeharia et al. 2009).

On the other hand, all nine patients harboring missense mutations survived: six Yemenite Jewish patients (p.Tyr77His/p.Tyr77His in five patients; p.Leu233Phe/p.Ala10Ser for one patient), one Arab patient

(p.Gly272Asp/p.Gly272Asp), and patients P2 and P3 reported here (p.Val279Met/p.Glu217Lys; p.Leu233Phe/ p.Leu233Phe).

However, two patients carrying at least one frameshift mutation recovered. One harbored a missense mutation p.Val279Met associated with a splicing mutation in the last intron c.1102-3C>G. Nevertheless, this splicing mutation may not be as harmful as those described in fatal cases since the aberrant transcript is not subject to nonsense-mediated mRNA decay and may be translated into a truncated protein that may keep a residual activity (p.Phe368SerfsX51) (Uusimaa et al. 2011). By contrast, mutations found in other patient were really deleterious: a frameshift mutation c.711_712insG (p.Gln238AlafsX14) and a nine-base–pair-in-frame insertion c.1081_ 1082insAGGCTGTGC (p.Arg361insAla,Val,Arg) close to a highly conserved glutamine involved in anticodon recognition (Kemp et al. 2011; Schara et al. 2011). It is worth noting that both patients received early coenzyme Q and carnitine supplementations; this could support mitochondrial functions and help to overcome the acute phase.

To sum up, in 14/16 complete genotypes described so far, patients carrying two missense mutations (except in first Met) seem to have better prognosis than patients carrying at least one frameshift or splicing mutation. Obviously, these observations between genotype and patients' outcome must be verified in a larger number of cases. However, these remarks could help evaluating prognosis or prenatal diagnosis considerations. Thus, a prenatal diagnosis was offered to P1 parents, due to clinical severity of index case. The second fetus was not carrying any mutation, the pregnancy continued and a healthy baby was born.

In conclusion, we report three unrelated cases of neonatal mitochondrial hepatopathies caused by *TRMU* mutations. The typical pattern associating deficiency of complexes I and IV and normal mtDNA copy number in liver is a strong argument for a TRMU deficiency. If liver biopsy is unavailable, we suggest that *TRMU* gene sequencing should be added to the first intention sequencing screening panel of early-onset mitochondrial liver diseases. Indeed, early molecular diagnosis could help to propose appropriate clinical managements that could perhaps facilitate remission. Further reports of additional patients with TRMU anomalies are necessary to fully elucidate prognostic factors.

One Sentence Take-Home Message

TRMU gene mutations can induce a mitochondrial liver disease in early infancy which outcome ranges from death to recovery.

Contributions of Individual Authors

GAIGNARD Pauline: identification of patients defect (biochemical analysis and genetic analysis), manuscript writing

GONZALES Emmanuel: diagnosis and follow-up of patient 3, part of the manuscript writing, revising manuscript

ACKERMANN Oanez: diagnosis and follow-up of patient 2, revising manuscript

LABRUNE Philippe: diagnosis and follow-up of patient 1, revising manuscript

CORREIA Isabelle: genetic analysis

THEROND Patrice: revising manuscript

JACQUEMIN Emmanuel: diagnosis and follow-up of patients 2 and 3, revising manuscript

SLAMA Abdelhamid: identification of patients' defect (biochemical analysis and genetic analysis), manuscript writing, and supervision

References

Chabi B, Mousson de Camaret B, Duborjal H, Issartel JP, Stepien G (2003) Quantification of mitochondrial DNA deletion, depletion, and overreplication: application to diagnosis. Clin Chem 49: 1309–1317

Coenen M, Antonicka H, Ugalde C et al (2004) Mutant mitochondrial elongation factor G1 and combined oxidative phosphorylation deficiency. N Eng J Med 351:2080–2086

Galmiche L, Serre V, Beinat M et al (2012) Toward genotype phenotype correlations in GFM1 mutations. Mitochondrion 12: 242–247

García-Cazorla A, De Lonlay P, Nassogne MC, Rustin P, Touati G, Saudubray JM (2005) Long-term follow-up of neonatal mitochondrial cytopathies: a study of 57 patients. Pediatrics 116: 1170–1177

Guan MX, Yan Q, Li X et al (2006) Mutation in TRMU related to transfer RNA modification modulates the phenotypic expression of the deafness-associated mitochondrial 12S ribosomal RNA mutations. Am J Hum Genet 79:291–302

Hagervall T, Edmonds C, McCloskey J, Björk G (1987) Transfer RNA (5-methylaminomethyl-2-thiouridine)-methyltransferase from Escherichia coli K-12 has two enzymatic activities. J Biol Chem 262:8488–8495

Kemp J, Smith P, Pyle A et al (2011) Nuclear factors involved in mitochondrial translation cause a subgroup of combined respiratory chain deficiency. Brain 134:183–195

Low E, Crushell E, Harty S, Ryan S, Treacy E (2008) Reversible multiorgan system involvement in a neonate with complex IV deficiency. Pediatr Neurol 39:368–370

Mandel H, Szargel R, Labay V et al (2001) The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. Nat Genet 29:337–341

Naviaux R, Nguyen KV (2004) POLG mutations associated with Alpers' syndrome and mitochondrial DNA depletion. Ann Neurol 55:706–712

Rustin P, Chretien D, Bourgeron T et al (1994) Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta 228:35–51

Sarzi E, Bourdon A, Chrétien D et al (2007) Mitochondrial DNA depletion is a prevalent cause of multiple respiratory chain deficiency in childhood. J Pediatr 150:531–534

Sasarman F, Antonicka H, Horvath R, Shoubridge E (2011) The 2-thiouridylase function of the human MTU1 (TRMU) enzyme is dispensable for mitochondrial translation. Hum Mol Genet 20: 4634–4643

Schara U, Von Kleist-Retzow JC, Lainka E et al (2011) Acute liver failure with subsequent cirrhosis as the primary manifestation of TRMU mutations. J Inherit Metab Dis 34:197–201

Spinazzola A, Viscomi C, Fernandez-Vizarra E et al (2006) MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. Nat Genet 38:570–575

Umeda N, Suzuki T, Yukawa M et al (2005) Mitochondria-specific RNA-modifying enzymes responsible for the biosynthesis of the wobble base in mitochondrial tRNAs. Implications for the molecular pathogenesis of human mitochondrial diseases. J Biol Chem 280:1613–1624

Uusimaa J, Jungbluth H, Fratter C et al (2011) Reversible infantile respiratory chain deficiency is a unique, genetically heterogenous mitochondrial disease. J Med Genet 48:660–668

Valente L, Tiranti V, Marsano RM et al (2007) Infantile encephalopathy and defective mitochondrial DNA translation in patients with mutations of mitochondrial elongation factors EFG1 and EFTu. Am J Hum Genet 80:44–58

Yan Q, Bykhovskaya Y, Li R et al (2006) Human TRMU encoding the mitochondrial 5-methylaminomethyl-2-thiouridylate-methyltransferase is a putative nuclear modifier gene for the phenotypic expression of the deafness-associated 12S rRNA mutations. Biochem Biophys Res Commun 342:1130–1136

Zeharia A, Shaag A, Pappo O et al (2009) Acute infantile liver failure due to mutations in the TRMU gene. Am J Hum Genet 85: 401–407