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Short Communication

Leigh syndrome associated with TRMU gene mutations

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ABSTRACT

tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase (TRMU) deficiency causes an early onset potentially reversible acute liver failure, so far reported in less than 30 patients. We describe two new unrelated patients with an acute liver failure and a neuroimaging compatible with Leigh syndrome (LS) due to TRMU deficiency, a combination not previously reported. Our report enlarges the phenotypical spectrum of TRMU disease.

1. Introduction

TRMU is a nuclear gene that encodes the mitochondrial-specific tRNA-modifying enzyme: 5-methylaminomethyl-2-thiouridylate methyltransferase. *TRMU* pathogenic variants were first reported as a modifier in patients with sensorineural deafness carrying homoplasmic m.1555G > A variant in the mitochondrial gene *MTRNR1* (OMIM *561000) [1]. In 2009, pathogenic variants in *TRMU* gene were described as responsible for acute liver failure in the first months of age (OMIM #613070) [2]. Patients who survived this life-threatening period fully recovered and had a normal development without recurrence. To our knowledge, 26 patients with *TRMU* pathogenic variants have been published. Since the first description of reversible hepatic failure, only a few other phenotypes with extra-hepatic manifestations have been reported. Leigh syndrome (LS), featured by hyperlactacidemia and symmetrical lesions in brainstem, basal ganglia and other brain structures, is the most common pediatric presentation of mitochondrial disease [3]. We present two recently diagnosed patients with acute liver failure caused by *TRMU* pathogenic variants associated to LS, a phenotypic combination not previously reported.

2. Case reports

We extensively searched the literature for patients with *TRMU* molecularly confirmed disease and clinical description. Supplementary Table 1 summarizes clinical, laboratory, radiological and genetic data described in TRMU patients in previously published patients (n = 26) and we added our two patients (Patient 1 is P27, Patient 2 is P28).

Patient 1, a girl, was the second child of non-consanguineous parents. She was born at term after an uneventful pregnancy. Her psychomotor development and growth parameters (weight, length and head circumference at +0.2 SD) were normal until the age of 3 months, when she presented a rapid deterioration and failure to thrive. She suffered progressive generalized hypotonia, fluctuating level of consciousness,

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decreased intake, weight-loss (−2 SD) and progressive hepatomegaly. Laboratory findings showed acute liver failure, metabolic acidosis and persistent high lactate in blood, urine and CSF. Brain MRI showed acute and symmetrical lesions of pontinus tracts, upper cerebellar peduncles, basal nuclei and white matter, with restriction on DWI and a lactate peak on MRS (See Fig. 1A-D). Abdominal ultrasound showed an increased size and echogenicity of the liver, without any other pathological findings. Echocardiography, EEG and ophthalmologic examinations were normal. The respiratory chain complex activities in muscle biopsy were decreased except for normal CII, and increased citrate synthase activity. Muscle biopsy findings suggested mitochondrial myopathy (see suppl table). Lactic acidosis correction and supplementation with carnitine, biotine and thiamine was initiated with transient clinical and biochemical improvement. After two months the patient was readmitted to PICU with severe hepatic failure, coagulopathy, tubulopathy and lactic acidosis, refractory to all treatments and she died at the age of 6 months.

Mitochondrial DNA sequencing was normal. Clinical whole exome sequencing (TruSight One®, Illumina, San Diego) demonstrated two mutations in *trans* in *TRMU* gene: c.160_161delTG:p.Cys54* (non-previously reported, but likely pathogenic according to ACMG criteria since it creates an stop codon) and c.680G > C:p.Arg227Thr (previously described in [4]). Parents were carriers of these variants.

Patient 2, a boy, was the first child of non-consanguineous parents. After a non-complicated pregnancy, he was born at term with low birthweight (−2.7 SD). During the first 12 h of life he presented generalized hypotonia with severe lactic acidosis, progressive elevation of transaminases and coagulopathy. Brain MRI showed T2 hyperintense supra and infratentorial lesions affecting bilateral thalami, subthalamic nuclei and brainstem and a lactate peak on MRS (See Fig. 1E-O). Further metabolic investigations showed high lactate/pyruvate ratio, important elevation of intermediate metabolites of Krebs cycle in urine, and hyperalaninemia. Under the suspicion of a mitochondrial disorder, treatment with coenzyme Q, riboflavin, thiamine and biotin were initiated with no clinical improvement. He showed a refractory hyperlactacidaemia (15–20 mmol/L) and died at the age of 25 days from a multiple organ failure. The respiratory chain complexes activities were measured in muscle and, except for CII, all of them and also citrate synthase activity were decreased. When analyzing their ratio, most of them became normalized, so no significant alterations were detected. The low activity of all complexes and also of citrate synthase, could indicate a low number of mitochondria in the muscle. Mitochondrial DNA sequencing was normal. Exome Sequencing showed two pathogenic variants in *TRMU*: c.2 T > A: p.Met1? (reported in [2]) and c.491delT: p.Leu164ProfsTer22 (non-previously reported, but also pathogenic according to ACMG rules due to frameshift change generating premature stop codon). Most relevant findings in patient's necropsy were: massive hepatic steatosis, increase in heart thickness at the expense of groups of vacuolated and granular cytoplasm myocytes, among which conserved myocytes were identified, and severe spongiotic vacuolization in thalamus, midbrain, trunk and focal cerebellum.

In summary, both patients presented an early onset Leigh syndrome and an acute liver failure with progression to multiorganic failure and fatal outcome due to TRMU variants, and can not be explained by other

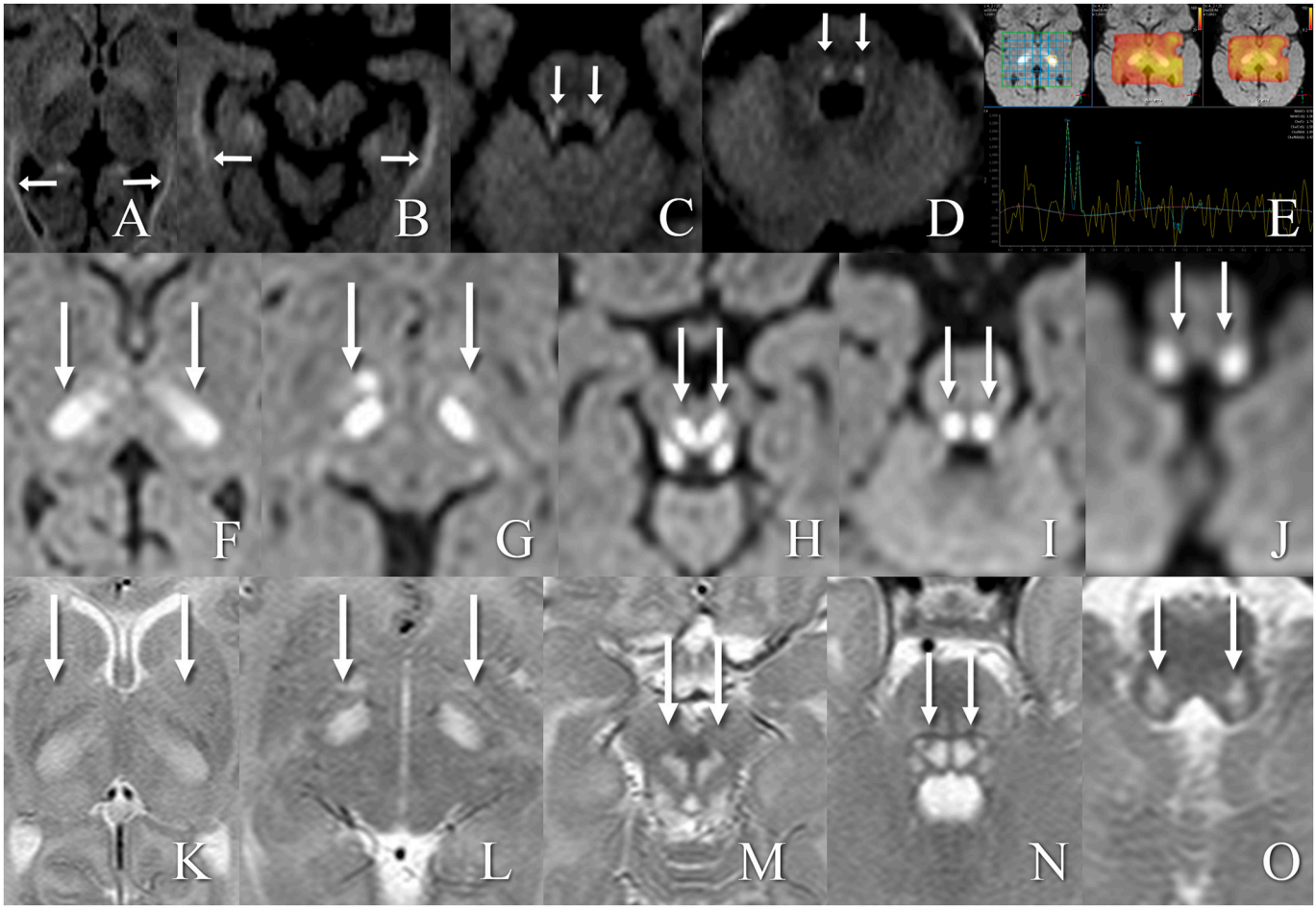

Fig. 1. MRI of patient 1 (P27 in the supplementary table) showed symmetrical lesions of pontinus tracts, upper cerebellar peduncles, basal nuclei and white matter with restriction in DWI (A-D). MRI of patient 2 (P28 in the suppl table) showed an increased lactate peak in the MRS (E), supra and infratentorial lesions affecting bilateral thalami, subthalamic nuclei and brainstem in DWI (F-J) and T2 (K-O).

genetic findings in the whole exome-sequencing.

3. Discussion

We report two recently diagnosed unrelated patients with *TRMU* pathogenic variants, associating LS and acute liver failure, a combination not previously reported.

Classical presentation of *TRMU* pathogenic variants has been described to date in 26 patients with acute liver failure as the main phenotype, some of which share other clinical findings (Supplementary table) [2,5–12]. Almost all (24/26) reported patients suffered from hepatic failure. One (P18) had a myopathic phenotype (ptosis, fatigability, bulbar involvement with feeding and respiratory difficulties at onset with complete recovery) and another (P20) a fatal heart failure in his first month of life. Other extrahepatic manifestations include a variable range of symptoms: reversible dilated cardiomyopathy and nephromegaly (P4), bulbar involvement with feeding difficulties (P18,P19), some degree of hypotonia (P15, P17, P18), hypothyroidism, macrocytic anemia and microcephaly (P21, P22), ichthyosis (P24), etc. Long-term outcome varies from complete recovery (19/26) to death due to liver, heart or multiorganic failure (7/26). When surviving the acute phase, most patients have a normal development and complete recovery. However, long term follow-up data are not available for all of these patients. Regarding biochemical findings, all patients showed increased serum lactate level (3.2–40.0 mmol/L), with variable range of lactate in CSF (from normal, to 3.9 mmol/L). Abnormal liver function was present in all patients (available data from 24/26) with variable degrees of transaminase elevation, coagulopathy, cholestasis or elevated alfa-fetoprotein. Liver biopsies (9/23) showed signs of mild to severe hepatic involvement in biopsies taken several months after onset: oncocytic changes in the hepatocytes, focal steatosis and focal ballooning of cytoplasm, micronodular cirrhosis, canalicular cholestasis to patent signs of fibrosis, irregular cirrhosis with nodulation and macrovesicular steatosis. No normal hepatic biopsies were reported. Analysis of respiratory chain enzyme activities demonstrated a combined respiratory chain complex deficiency in most of the cases with available data (11/26). Brain MRI was performed in 17/26 patients. All except three were normal. P16 had a lactate peak in MRI with no lesions, P15 had myelination delay with normal MRS and P19 had a reversible T2 high signal in right thalamus with lactate peak in MRS.

Our patients are similar to the ones reported in literature in terms of liver failure and abnormal laboratory findings. In contrast, our patients presented a neurological clinical onset consisted of an acute encephalopathy and developmental regression (P1) and severe hypotonia from birth (P2). All two displayed symmetrical and bilateral lesions in MRI affecting different brain structures consistent with LS, with a lactate peak on MRS. Postmortem study in P2 demonstrated massive hepatic steatosis, also seen in liver biopsies of previous patients and spongiotic vacuolization in thalamus, midbrain, trunk and focal cerebellum as seen in LS [3]. Interestingly, this necropsy showed cardiac affection, despite the patient not having presented evident cardiological symptoms. Only two previous patients (P4, P20) were mentioned to have cardiological involvement.

Clinical presentation of Leigh syndrome varies depending on the age of onset [13]. In typical infantile onset, initial nonspecific manifestations can be seen: vomiting, hypotonia, failure to thrive or developmental delay, often with acute worsening of previous clinical state after a clinical event such as infection. On the other hand, liver involvement it is a very unusual clinical manifestation. Bilateral symmetrical lesions within the brainstem, basal ganglia and less frequent white matter, T2-W in MRI characterize the disease with elevated lactate in MRS [14]. Both clinical and neuroimaging features occurred in our two patients. Pathogenic variants in over 75 genes (nuclear and mitochondrial DNA), have been associated to Leigh syndrome [14], despite what, half of the cases remain without a genetic diagnosis [13]. Defects of nuclear DNA genes are the main cause of Leigh syndrome, with only 25% of all cases caused by mtDNA pathogenic variants. Both isolated and multiple complex deficiencies can be found, being these last ones mainly caused by mutations in more than 150 proteins implicated in mitochondrial translation, including TRMU [15]. Axial and/or peripheral hypotonia was described in four patients (P15, P16, P18 and P19), P25 had seizure-like episodes and mild developmental delay has been reported in P24 and P26. Although neurological involvement has been described in some patients, none of them had brain lesions compatible with Leigh syndrome.

TRMU encodes a protein that participates in the modification of mitochondrial tRNAs. Specifically, it is responsible for the 2-thiolation of the nucleotide at the wobble position of the tRNA-Lys, tRNA-Glu, and t-RNA-Gln [2]. Many other genes related to mitochondrial translation are identified to cause Leigh syndrome [13,14]. Similar pathogenic mechanisms could be shared between these genes to explain Leigh syndrome in our patients, but further investigations are needed to understand why only a few patients with *TRMU* pathogenic variants developed this phenotype.

Although most of the reported patients (19/26) experienced a partial or total recovery after the acute phase, all of our patients died during the first months of life. They presented severe neurological involvement and finally died from multiorganic failure.

A possible explanation to clarify a genotype-phenotype correlation has been proposed [2]: patients carrying two missense variants (except those occurring in first methionine) seem to have better prognosis than when, nonsense, frameshift or splicing variants are present. Both patients carried nonsense and frameshift variants respectively, in compound heterozygosity with known missense pathogenic variants. On the other hand, first descriptions of TRMU defects suggested increasing cysteine availability in later infancy as a possible explanation for clinical recovery [2]. Patient 2 had an abnormal birth weight, which may induce thinking that cysteine deficiency in maternal and neonatal nutrition could also be a potential contributor to a severe phenotype. However, patient 1 had normal weight before clinical deterioration at 3 months old. Therefore, we conclude that neither molecular findings nor maternal/neonatal nutrition seem reason enough to explain the clinical heterogeneity of the disorder.

To conclude, *TRMU* gene sequencing should be considered in patients with Leigh syndrome, especially when liver involvement is present, and also in patients with other mitochondrial phenotypes as progressive failure to thrive, mild-moderate psychomotor delay, mild liver involvement and metabolic acidosis with hyperlactacidemia. Further patient reports and more investigations are needed to explain this phenotypical variability even though carrying same pathogenic variants. Our report enlarges the phenotypical spectrum of TRMU disease.

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Author statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before this submission.

Authorship contributions

Specific author contributions are listed below.

- Júlia Sala-Coromina: Conceptualization, methodology, visualization, writing – original draft.
- Lucía Dougherty-de Miguel: Conceptualization, methodology, visualization, writing – original draft.
- Javier de las Heras: Conceptualization, methodology, writing.
- Amaia Lasa-Aranzasti: Methodology, data curation and review.
- Elena Garcia-Arumi: Methodology, supervision, writing review.
- Lidia Carreño: Methodology, data curation
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- Clara Carnicer: Methodology, data curation.
- María Unceta-Suárez: Methodology, data curation.
- Angel Sanchez-Montañez: Methodology, writing review. and editing.
- Laura Gort: Methodology, data curation, writing review.
- Frederic Tort: Methodology, writing review.
- Mireia del Toro: Conceptualization, methodology, supervision, writing review and editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2020.100690.

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