A novel TRMT5 mutation causes a complex inherited neuropathy syndrome: The role of nerve pathology in defining a demyelinating neuropathy

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Abstract

**Aims:** We aim to present data obtained from three patients belonging to three unrelated families with an infantile onset demyelinating neuropathy associated to somatic and neurodevelopmental delay and to describe the underlying genetic changes.

**Methods:** We performed whole-exome sequencing on genomic DNA from the patients and their parents and reviewed the clinical, muscle and nerve data, the serial neurological studies, brain and muscle MRIs, as well as the respiratory chain complex activity in the muscle of the three index patients. Computer modelling was used to characterise the new missense variant detected.

**Results:** All three patients had a short stature, delayed motor milestone acquisition, intellectual disability and cerebellar abnormalities associated with a severe demyelinating neuropathy, with distinct morphological features. Despite the proliferation of giant...
mitochondria, the mitochondrial respiratory chain complex activity in skeletal muscle was normal, except in one patient in whom there was a mild decrease in complex I enzyme activity. All three patients carried the same two compound heterozygous variants of the TRMT5 (tRNA Methyltransferase 5) gene, one known pathogenic frameshift mutation [c.312_315del (p.Ile105Serfs*4)] and a second rare missense change [c.665 T > C (p.Ile222Thr)]. TRMT5 is a nuclear-encoded protein involved in the post-transcriptional maturation of mitochondrial tRNA. Computer modelling of the human TRMT5 protein structure suggests that the rare p.Ile222Thr mutation could affect the stability of tRNA binding.

Conclusions: Our study expands the phenotype of mitochondrial disorders caused by TRMT5 mutations and defines a new form of recessive demyelinating peripheral neuropathy.

KEYWORDS
inherited neuropathy, mitochondrial disorders, mitochondrial neuropathies, TRMT5

INTRODUCTION

Charcot–Marie–Tooth disease (CMT) has long been recognised as a heterogeneous group of inherited neuropathies. Peripheral neuropathy can either be the dominant feature of a condition (primary neuropathy) or be part of a more complex syndrome. Moreover, these complex neuropathies can be further classified into two main types of syndromes: a purely neurological syndrome associated with dysfunctions in both the central and peripheral nervous systems (CNS and PNS); and a second group combining neurological and non-neurological abnormalities. Some complex neuropathies have a congenital and ‘syndromic’ presentation, and they are regarded as developmental rather than degenerative disorders as they are caused by inherited metabolic dysfunctions. Among the metabolic diseases associated with a peripheral neuropathy, a group of mitochondrial disorders is characterised by dysfunction in pathways involved in mitochondrial oxidative phosphorylation, provoking oxidative stress. These disorders may be the result of mutations in maternally inherited mitochondrial DNA (mtDNA) or nuclear DNA (nDNA), genes encoding proteins responsible for mitochondrial gene expression.

There is growing evidence that some neurological diseases are associated with mutations in the nuclear genes involved in the mitochondrial transcriptome. The mtDNA encodes for 22 transfer RNAs (mt-tRNAs) that can each undergo post-transcriptional modifications. The tRNA methyltransferase 5 (TRMT5) is a nuclear gene (MIM*611023) encoding a protein that catalyses methylation at the N1 position of guanosine at residue 37 (G37) of various mitochondrial tRNAs, a modification necessary to enhance translational efficiency. In three separate families, mutations in this gene have been associated with a series of clinical defects, including exercise intolerance, neuropathy, spasticity, developmental delay and deficient mitochondrial respiratory chain (MRC) complex I and IV activity in skeletal muscle. Here, we describe the detailed phenotype of three apparently unrelated patients who carry compound heterozygous mutations in the TRMT5 gene, each of whom developed a complex neuropathic syndrome that affects the CNS and peripheral nerves. Electron microscopy of the nerves was key to reaffirm that these mutations produce a demyelinating neuropathy.

MATERIALS AND METHODS

Patients

We investigated three apparently unrelated families of Southern European descent in which healthy non-consanguineous parents have two progenies (see pedigree in Figure 1). All direct members of these families reflected in the pedigrees were subjected to a detailed neurological examination by the same experienced neurologist (TS). Cognitive features were collected from screening tests and clinical interviews and from testing by school authorities, although no formal cognitive testing was carried out at our clinic. In the three probands,
A NOVEL TRMT5 MUTATION CAUSES A COMPLEX INHERITED NEUROPATHY

FIGURE 1

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mutations in known genes associated with inherited peripheral neuropathies had already been ruled out using a neuropathy-associated gene panel. The patients have been followed up at our institution from early childhood and serial nerve conduction studies (NCS) were available for review. Several brain and whole-body muscle MRI scans were performed on the three patients.

Genetic analysis

A genetic diagnosis of the three unrelated probands and their healthy parents was made after performing Next Generation Sequencing (NGS) driven whole exome sequencing (WES). Capture-based exome enrichment was carried out using a Human Exome Capture tool (CSP, v5, Agilent technologies, Santa Clara, CA, USA) and libraries were sequenced using an Illumina HiSeq 2000 platform at the CNAG (Centro Nacional de Análisis Genómico). The WES pipeline at the CNAG was used for variant identification and annotation, and data analysis was performed on the RD-Connect Genome Phenome Analysis Platform (https://platform.rd-connect.eu/genomics/) applying standard criteria for a rare disease. Validation of the variants identified and segregation studies on family members was performed by Sanger sequencing. Kinship analysis was performed as a quality control of sample identity and to confirm that the families were unrelated. TRMT5 variants were screened by NGS in a cohort of 20 children and 96 adults with CMT but without a genetic diagnosis.

Multiple mtDNA deletions in DNA from patients' muscle biopsies were analysed by long range polymerase amplification of the whole mtDNA molecule using the primers pair 5′-CCGGACAGGAGGCTACCTCCTCC-3′ and 5′-GATATTGATTTCACGGAGGATGGTG-3′ and the SequalPrep™ Long PCR Kit (ThermoFisher Scientific, MA, USA) and 19 common point mtDNA mutations (m.3243A > G; m.3460G > A; m.8344A > G; m.8993 T > G/T > C; m.9176 T > C/T > G; m.10158 T > G; m.10191 T > C; m.13513G > A; m.13514A > G; m.11777G > A; m.11778G > A; m.11832G > A; m.14459G > A; m.14482C > A/C > G; m.14484 T > C; m.14487 T > C) were analysed in DNA from patients’ skeletal muscle by minisequencing-SNaPShot Multiplex (ThermoFisher, Applied Biosystems).10

Protein alignment and structural modelling

To gain insight into the protein conservation across different species, we performed a multiple alignment of TRMT5 proteins using the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/). The 3D structural model of human TRMT5 was generated using the Swiss-model server (https://swissmodel.expasy.org/) and based on the crystal structure of Methanocaldococcus jannaschii TRMT5 complexed with tRNACys and S-adenosylmethionine (SAM; PDB - 2ZNN).

Muscle and nerve biopsies

Open muscle biopsies of the tibialis anterior (F1/II:2), deltoid (F2/II:2) and quadriceps (F3/II:2) muscles were obtained for histopathological and biochemical analysis. Samples were snap frozen or fixed and embedded in appropriate material for electron microscopy. Transverse cryo-sections were processed by routine histological and histochemical techniques for a light microscopy evaluation of their morphology, whereas ultrathin cuts from plastic blocks were analysed by conventional electron microscopy.11 The MRC and citrate synthase enzyme activities were determined spectrophotometrically in the skeletal muscle homogenates using standard methods, with minor modifications.12 A sural nerve specimen from patient F3/II:2 at 8 years of age and patient (F1/II:2) at 9 years of age were analysed by light and electron microscopy, as described previously.13

Standard protocol approvals, registrations and patient consent

This study was approved by the Hospital Universitari i Politècnic La Fe ethics committee, and written informed consent was obtained from the probands’ guardians prior to commencing the study, including consent for publication and to disclose recognisable persons in a figure.

RESULTS

Clinical features

The clinical characteristics of the three patients are summarised in Table 1, each from a family with no history of neuromuscular disorders. The clinical assessment of the parents and unaffected siblings was normal, as were the results of the electrophysiological studies on the parents of patient F1/II:2. Congenital global developmental delay in motor, speech, cognitive and social areas was detected in the three...
| Patient age (sex) | Onset | Age at onset | Early features | Functional status (age) | Pattern of muscle weakness on last exam | UMN features | Cerebellar features | Intellectual disability | Additional features |
|------------------|-------|--------------|----------------|-------------------------|----------------------------------------|-------------|--------------------|----------------------|----------------------|
| F1/II:2 | Birth | Podalic presentation, delayed motor milestones (walking at 24 m) | AFOs (9 years), walker (12 years) and wheelchair most of the time (14 years) | – – + ++ +++ | Bilateral Babinski sign and brisk palomental reflexes | Saccadic EM, dysmetria and dysdiadochokinesis in UL, gait ataxia | Severe ID (starts reading aged 9 years), inattentive disorder, attends special needs school (14 years) | Podalic presentation, severe growth impairment treated with GH replacement therapy (3 years), severe scoliosis. Severe ATT, pes cavus with hammer toes. |
| F2/II-2 | 10 m | Delayed motor milestones (sitting was unstable at 10 m) | AFOs (34 m), walker (6 years) and wheelchair (6 years) | – ++ +++ +++ | Bilateral Babinski sign. | Gait ataxia, he did not collaborate enough for further evaluation | Severe ID (limited receptive and expressive language), special needs school (6 years) | Speech and swallowing difficulties suggesting bulbar dysfunction (8 years), poor sphincter control, febrile seizures, growth impairment, moderate scoliosis (15 years). Elbows, claw hand, knees, severe ATT, pes planus with laterally deviated first toes. |
| F3/II:2 | 19 m | Delayed motor milestones (walking at 28 m) | AFOs (6 years), and wheelchair for long distances (7 years) | – + ++ +++ | Bilateral Babinski sign | Saccadic EM, moderate dysmetria in UL, gait ataxia | Mild to moderate ID (speech delay aged 3 years), attention deficit and hyperactivity disorder on medication (6 years), mainstream class with extra teaching support | Growth impairment, lumbar hyperlordosis (3 years), no scoliosis. Moderate ATT, pes planus. |

Abbreviations: F, female; M, male; UL, upper limb; prox, proximal; LL, lower limb; UMN, upper motor neuron; m, months; y, years; –, absent; +, mild; ++, moderate; ++++, severe; AFO, ankle-foot-orthosis; ATT, Achilles tendon tightening; EM, eye movements; GH, growth hormone; ID, intellectual disability.
Patient F1/II:2 was a 17-year-old female who was born by caesarean section due to a podalic presentation. She started walking unassisted at 24 months of age, with frequent falls. At the age of 3, she was diagnosed with growth delay associated with growth hormone deficiency, receiving replacement therapy until she was 14. She was attended by a paediatric neurologist from the age of 4 years due to speech and motor delay, and a sensorimotor demyelinating neuropathy was identified at this point. At 9 years of age, she was diagnosed with attention deficit with no hyperactivity and with learning difficulties, and she was treated with methylphenidate for a year with no response. She was referred to our neuromuscular clinic aged 7, and a clinical examination showed distal muscle weakness in the lower limbs (ankle dorsiflexion was grade 3 on the Medical Research Council scale), an absence of deep tendon reflexes, bilateral Achilles contractures, pes planus and tiptoe walking. She was not collaborative enough to assess sensation, but she was unstable with her feet together and fell immediately after closing her eyes. She had mild dysmetria on finger-nose testing, hypotonia, extensor plantar responses and her slow pursuit eye movement was saccadic. She needed bilateral support to walk and her gait was ataxic, with a severe foot drop. She developed scoliosis that required surgery at 14 years of age. From this age, she attended a special needs school and used a wheelchair to travel over short distances. After a 10 year follow-up, the clinical examination at the age of 17 revealed short stature (142 cm, <3rd percentile), mild intrinsic hand muscle weakness without atrophy, bilateral extensor plantar responses, bilateral palmar reflexes and hypotonia. Vibration was diminished in the upper and lower limbs in a length-dependent manner and position sense was abolished at both halluces.

Patient F2/II:2 was a 15-year-old male. Pregnancy and delivery were uneventful (Apgar 9/10), yet motor development was delayed and he could not sit without support at 10 months of age. At 19 months of age, he was still unable to walk and NCS were compatible with a demyelinating sensory-motor neuropathy. He began walking at the age of 34 months, assisted with an ankle-foot orthosis (AFO), although he was unable to stand until the age of 6 using a frame walker and he had to use a wheelchair over long distances. He experienced several complex febrile seizures throughout his childhood, from the age of 14 months until 7 years of age, yet he was never given antiepileptic medication. At the age of 6 he had poor sphincter control and still used diapers, and he was unable to read or form a five-word sentence. He was evaluated at our unit when he was 7 years old, revealing severe weakness and atrophy in the lower limbs and hands, with severe ankle and knee tendon retraction that prevented him from staying upright. Deep tendon reflexes were absent and his toes were upturned bilaterally. Sensitivity could not be assessed due to difficulties in understanding orders, and no nystagmus or cranial nerve involvement was observed. Aged 8, he developed dysphagia to liquids that required a change in the consistency of his diet ever since. At the last evaluation, at age 15, his speech was limited and his comprehension of simple commands was deficient.

Physical examination showed growth impairment (120 cm, <3rd percentile), limited movement of the ankles, weakness of intrinsic hand muscles and multiple contractures in the elbows, wrists, fingers, knees and ankles.

Patient F3/II:2 was a 9-year-old boy who started walking at 28 months of age but experienced frequent falls. Neurological examination at the age of 3 showed pes planus, areflexia, lumbar hyperlordosis and instability when walking, and he was unable to heel walk. A neurophysiological study at that time revealed a demyelinating sensorimotor peripheral neuropathy, and he still did not speak clearly at that age, only putting two words together and unable to form sentences. Handling small objects had always been difficult for him, and at the age of 6, he was diagnosed with attention deficit and hyperactivity disorder, requiring extra teaching support in a mainstream class. He has been wearing AFOs since he was 6 years old and needed a wheelchair to travel over longer distances. Clinical examination at the age of 9 revealed weakness of foot dorsiflexion, leg atrophy below the knees and mild intrinsic hand muscle weakness with thenar eminence atrophy. Deep tendon reflexes were absent, and although muscle tone was normal, he had bilateral extensor plantar responses. Cerebellar effects were manifested, with saccadic eye movements during slow pursuit and dysmetria on finger-nose testing. His gait was markedly ataxic and growth impairment with bone age delayed by 2 years was confirmed. He was 111 cm tall (<3rd percentile).

In the three patients, appropriate ancillary testing and examinations excluded any visual, auditory, renal, liver, gastrointestinal, or primary cardiac abnormalities. ECGs and echocardiograms were normal in all patients, as was 24 h Holter monitoring in patient F2/II:2. Targeted metabolic work-up did not identify any inborn errors in metabolism. Serum growth hormone level was within normal limits for patients F2/II:2 and F3/II:2. Serum and urine lactate levels were normal for patients F3/II:2 and F1/II:2 (5 serial measurements over a 7-year period), whereas they were mildly elevated in patient F2/II:2 (serum 3.30 mmol/L [n.c. <2.2], urine 642 mmol/L [n.c. <107]).

**Neurophysiology**

All patients were subjected to serial electrophysiological studies (Table 2). Sensory nerve action potentials (SNAPs) were not evident in any patient from the first time they were tested. Moreover, motor nerve conduction velocities (MNCVs) were reduced to values between 20 to 35 m/s, and the cortical magnetic motor-evoked potential (MEP) to the lower and upper limbs was prolonged in the two patients in whom it was measured (F2/II:2 and F3/II:2).

**Brain and muscle MRI**

Cranial MRI findings highlighted a variable degree of vermal and hemispheric cerebellar atrophy, which was more prominent in the
Regarding the ... to be altered by substituting Ile222 with a polar residue like Thr.

**Genetic findings**

The WES analysis of the three unrelated families identified two compound heterozygous variants in the TRMT5 gene: [NM_020810.3: c.312_315del; NP_065861.3: p.Ile105Serfs*4] and [NM_020810.3: c.665 T > C; NP_065861.3: p.Ile222Thr]. Although the c.312_315del change is annotated in the Single Nucleotide Polymorphism (SNP) database (rs755184077), with 246 heterozygotes out of 282,782 allele counts in the Genome Aggregation Database (gnomAD, accessed 27 October 2021), no homozygotes were reported in healthy controls. Indeed, this mutation was previously described as pathogenic in trans with other missense mutations. Regarding the second change identified in trans, c.665 T > C, this was also present in the SNP database (rs766935145) but with only one heterozygote out of 241,828 allele counts in gnomAD (accessed October 27th, 2021). This variant is not predicted to create a cryptic splice site according to spliceAI. Segregation analysis in healthy siblings confirmed that both mutations segregated with the disease in an autosomal recessive pattern of inheritance (Figure 1). No additional patients carrying TRMT5 pathogenic variants were identified in the cohort screened. The presence of multiple mtDNA large deletions and 19 common mtDNA point mutations were excluded in skeletal muscle.

**In silico pathogenic studies**

A multiple sequence alignment (MSA) of the TRMT5 protein sequence showed that the mutated Ile222 amino acid residue is highly conserved among species (Figure 1). To shed light on the impact of the new p.Ile222Thr mutation on protein activity, we analysed the 3D structural model of human TRMT5 generated using the structure of the TRMT5 from *M. jannaschii* bound to tRNA Cys and SAM (PDB ID 2ZNN) as a template. Ile222 is located in the D2 domain of TRMT5, a domain that participates in tRNA binding and in catalysis. Although Ile222 is outside the tRNA modification site, structural analysis indicates that as in MtTRMT5, Ile222 (equivalent residue in MtTRMT5, Ile120) is located in a hydrophobic environment of human TRMT5, stabilising the folding of the D2 domain that would be expected to be altered by substituting Ile222 with a polar residue like Thr.
Muscle biopsy findings

Routine muscle histochemistry in proximal muscle samples identified normal tissue or only minor abnormalities, such as a predominance of type I muscle fibres in both the proximal muscle biopsies (case F2/II:2, corresponding to Figure 4B, and patient F3/II:2). By contrast, in the tibialis anterior tissue, there were signs of chronic denervation, such as fibre-type grouping (case F1/II:2). Both modified Gomori trichrome staining and oxidative reactions (DPNH-TR and SDH) revealed a slight reinforcement of the intermyofibrillar network, yet there was no striking subsarcolemmal accumulation indicative of ‘ragged red fibres’. Moreover, neither cytochrome oxidase negative fibres nor lipid droplets were apparent following oil-red stain. The ultrastructural examination revealed abundant chains of large mitochondria occupying most of the inter-myofibrillar spaces. In addition, the mitochondrial shape and the structure of the cristae was preserved, and no abnormal internal deposits or crystalline structures were visible (Figure 4). Enzymatic analysis of the MRC returned normal values, except for a mild single complex I deficit in patient II:2 from family 1 (additional data are in Table S1).

Nerve biopsy

Light microscopy examination of semi-thin transverse sections showed a mild (Figure 4, case F2/II:2) or moderate loss of myelinated fibres (Figure 4, case F3/II:2). The remaining fibres were of small or intermediate diameters. A high proportion of the myelinated fibres from case F3/II:2 (around 60%) or a smaller proportion in case F2/II:2 (20%) presented disproportionately thin or thick myelin sheaths in relation to the axon calibre, or they featured irregular myelin shapes (Figure 4). The perineurium, endoneurium and blood vessels appeared normal. Electron microscopy depicted a wide variety of myelin abnormalities in the images obtained from the two nerve biopsies: hypo-myelinated fibres (Figure 4), split and uncompact myelin lamellae (Figure 4), and focal myelin infolding or outfolding (Figure 4).

Small hypomyelinated fibres were highly abundant in case F3/II:2, and often appearing as axons enclosed by very thin myelin sheath or a few uncompacted lamellae (Figure 4), thus giving the impression of a delayed or arrested myelinization at initial stages. The Schwann cells associated with these immature fibres displayed profuse cytoplasm, and they often develop small supernumerary and elongated extensions; in any case neither bulbs of concentric Schwann cell processes nor those of empty basal lamina were observed. In general, axon structure was well preserved but large mitochondria were often seen in the axoplasm (Figure 4). Furthermore, large mitochondria were also observed in the Schwann cell cytoplasm (arrowhead, Figure 4). Otherwise, apart from their abnormal size, the peripheral nerve mitochondria seldomly presented structural abnormalities.

FIGURE 2 Brain MRI findings in the three children with mutations in the TRMT5 gene. (A, B) MRI study of patient F1/II:2 (aged 17 years) showing cerebellar hemispheric atrophy (A) and scattered foci in the subcortical white matter (white arrowheads) of the parietal (A) and frontal (B) regions. (C, D) Axial T2-weighted imaging corresponding to patient F2/II:2 (aged 14) that shows increased CSF in the cerebellar folia of the upper cerebellum (C) and foci in the subcortical white matter (white arrowheads) of the frontal lobe (D). (E–G) T1-weighted midsagittal views from patient F2/II:2 (aged 14; E) and individual F3/II:2 (aged 8 years; F, G) showing moderate vermian atrophy that mainly involves the anterior lobe of the vermis, folium vermis (Fo) and tuber vermis (tu), along with moderate atrophy of the cerebellar tonsil (Cl).
We have identified a rare haplotype in TRMT5 associated with demyelinating polyneuropathy in three apparently unrelated families. Peripheral neuropathy and intellectual disability were the predominant features in our patients, in whom additional findings included cerebellar ataxia, pyramidal signs and short stature. One of the patients also suffered from complex febrile seizures for which medication was not given. The demyelinating neuropathy was predominantly sensory from the outset, as witnessed by the absence of SNAPs in all the neurophysiological studies carried out on the patients.

Recessive mutations in the TRMT5 gene have been reported previously in three families who share the pathogenic c.312_315del frameshift mutation with our families, a deletion that produces a premature stop codon p.Ile105Serfs*Ter4. However, the clinical presentation in these earlier cases was notably different, featuring exercise intolerance, lactic acidosis and evidence of multiple MRC deficiencies in skeletal muscle. There was relative sparing of the anterior and posterior tibialis muscles in F1/II:2 (G). T1-weighted axial images of the calf (G-I) showing prominent fatty replacement of the peroneus longus muscle, and a lesser and variable degree of fatty replacement in the solei and medial gastrocnemius muscles. There was relative sparing of the anterior and posterior tibialis muscles in F1/II:2 (G).

Demyelinating neuropathy and intellectual disability can also occur as a result of mutations in other genes that affect mitochondrial dynamics, such as SURF1, MMF and PTHR2. Patients with SURF1 defects generally display gait ataxia, growth failure, developmental regression, lactic acidemia and sensorimotor neuropathy, either axonal or demyelinating (Leigh syndrome). However, two families with severe childhood-onset neuropathy with a MNCV <25 m/s and lactic acidemia have been described. Mutations in the MFF gene have also been associated with Leigh syndrome like encephalopathy, optic atrophy, spasticity and cerebellar atrophy. Although these patients also presented with a congenital demyelinating neuropathy, a fuller comparison with our patients is not possible as the MFF neuropathy was not described in detail.

In addition, recessive mutations in the MCM3AP gene have been reported as a cause of childhood onset severe sensorimotor neuropathy, intellectual disability and MRI abnormalities in some patients. The

**FIGURE 3** Lower limb muscle MRI of the three children carrying mutations in the TRMT5 gene. Left column (A, D, G, J) corresponds to patient F1/II:2 at 17 years of age, the middle column (B, E, H, K) corresponds to F2/II:2 aged 14, and the right column (C, F, I, L) represents the individual F3/II:2 at 8 years of age. T1-weighted axial images of the upper (A-C) and lower thigh (D-F) showing an overall loss in volume. T1-weighted axial images of the calf (G-I) showing prominent fatty replacement of the peroneus longus muscle, and a lesser and variable degree of fatty replacement in the solei and medial gastrocnemius muscles. There was relative sparing of the anterior and posterior tibialis muscles in F1/II:2 (G). T1-weighted axial images of the foot (J-L) showed atrophy and diffuse fat infiltration of the flexor plantar muscles without complete fat replacement.

**DISCUSSION**

We have identified a rare haplotype in TRMT5 associated with demyelinating polyneuropathy in three apparently unrelated families. Peripheral neuropathy and intellectual disability were the predominant features in our patients, in whom additional findings included cerebellar ataxia, pyramidal signs and short stature. One of the patients also suffered from complex febrile seizures for which medication was not given. The demyelinating neuropathy was predominantly sensory from the outset, as witnessed by the absence of SNAPs in all the neurophysiological studies carried out on the patients.

Recessive mutations in the TRMT5 gene have been reported previously in three families who share the pathogenic c.312_315del frameshift mutation with our families, a deletion that produces a premature stop codon p.Ile105Serfs*Ter4. However, the clinical presentation in these earlier cases was notably different, featuring exercise intolerance, lactic acidosis and evidence of multiple MRC deficiencies in skeletal muscle. Variable clinical findings included cardiomyopathy and a failure to thrive. Some of these patients developed neuropathies after decades of evolution, yet never was neuropathy the main feature of their syndrome. The cases presented here match some aspects of those previously described phenotypes; however, our patients did not display exercise intolerance or a biochemical phenotype suggestive of an OXPHOS abnormality and no COX-negative muscle fibres were identified. As MRC enzyme analysis may not always be abnormal or diagnostic, deep clinical phenotyping is essential in suspected mitochondrial neuropathies. This is illustrated by the clinical and biochemical comparison of our patients with the four subjects described previously (see Table 3).

Demyelinating neuropathy and intellectual disability can also occur as a result of mutations in other genes that affect mitochondrial dynamics, such as SURF1, MMF and PTHR2. Patients with SURF1 defects generally display gait ataxia, growth failure, developmental regression, lactic acidemia and sensorimotor neuropathy, either axonal or demyelinating (Leigh syndrome). However, two families with severe childhood-onset neuropathy with a MNCV <25 m/s and lactic acidemia have been described. Mutations in the MFF gene have also been associated with Leigh syndrome like encephalopathy, optic atrophy, spasticity and cerebellar atrophy. Although these patients also presented with a congenital demyelinating neuropathy, a fuller comparison with our patients is not possible as the MFF neuropathy was not described in detail. Recessive mutations in PTHR2 have been linked to an infantile multisystem disorder that included demyelinating sensorimotor neuropathy, sensory neuronal hearing loss, cerebellar hypoplasia and exocrine pancreatic failure. However, mutations in these genes produce many systemic alterations and neuropathy is not one of the main characteristics of the phenotype.

In addition, recessive mutations in the MCM3AP gene have been reported as a cause of childhood onset severe sensorimotor neuropathy, intellectual disability and MRI abnormalities in some patients. The
neuropathy in these patients was predominantly axonal, with a severe decrease in CMAP amplitudes, which contrasts with the severe involvement of sensory nerves in our patients. In some of the former cases, mild non-specific increases in signal intensities were found in T2-weighted brain MRI images. Recently, a mutation in the gene encoding the mt-tRNA^Val^ was associated with CMT in a large Venezuelan family. Muscle analysis revealed mitochondrial hyperplasia and a mild increase in glycogen, with a preserved mitochondrial morphology.

The pathological analysis in our patients’ nerves revealed a profound abnormality of the myelination cascade with alterations at different stages from the initiation of the process to the phase of lamellae compaction and the regulation of the thickness and shape of the myelin sheath. These features have not been thoroughly analysed in the other reported mitochondrial demyelinating neuropathies; thus, we cannot conclude whether these abnormalities are specifically associated with the TRMT5 mutations harboured by our patients. Though we observed shared features with diverse CMT de-or-dys-myelinating...
| Reference | Patient ID-sex | TRMT5 variants | RCC deficiency | Histochemical COX defect/lactic acidosis | Age at onset | Clinical picture | NCS results | Neuroimaging findings |
|-----------|----------------|----------------|----------------|----------------------------------------|--------------|-----------------|-------------|---------------------|
| Powell CA, Kopajtic R, DSouza A, et al. | 73,901-F | c.[312_315del;872G > A], p.[ile105Serfs*4;Arg291His] | I, II + III, and IV | >95% COX deficient fibres/Yes | Childhood | Exercise intolerance, dyspnoea, spasticity/peripheral neuropathy | Needle EMG and motor NCV normal (aged 27 years). | NA |
| Tarnopolsky M, Brady L, Tetreault M, Consortium F. | 65,205-M | c.[312_315del;1156A > G], p.[ile105Serfs*4;Met386Val] | I and IV | None | Birth | Global development delay, neuropathy. Severe intellectual disability. Systemic involvement (neonatal period): hypertrophic non-obstructive cardiomyopathy, dysmorphic signs, gastrointestinal dysmotility, growth impairment. | NCS (17mo) demyelinating neuropathy. | Brain MRI: slight brain atrophy, a larger left hemisphere, and delayed myelination. |
| Present work | F1/II-2-F, F2/II-2-M, F3/II-2-M | c.[312_315delAATA; 749 T > C], c.[312_315del;872G > A], p.[ile105Serfs*4;Arg291His] | I in F1/II-2 | No COX-negative fibres/No (only) | Birth, infantile | Early-onset neuropathy. Cerebellar impairment. Pyramidal tract involvement. | Sensory predominant demyelinating neuropathy. | Brain MRI: cerebellar atrophy. |

(Continues)
neuropathies particularly those associated with congenital hypomyelinating phenotypes, such as PMP, MZP and others, our cases differ from many of them by the absence of common features like onion bulbs or basal lamina reduplication.

Although mutations in TRMT5 impair proper mitochondrial translation, leading to a general defect of mtDNA encoded proteins and eliciting a combined defect of MRC complex activities, only a single complex I deficiency was detected in skeletal muscle from one of the three probands. In muscle biopsies, three of four previous TRMT5 patients had variable defects in MRC complexes that were associated with altered mitochondrial histology/morphology (Table 3). MRC enzyme activity was not evaluated in one of these patients due to the absence of histological abnormalities. Interestingly, no COX staining abnormalities in the muscle were detected in the three patients reported here, even though an abnormal ultrastructure of mitochondria was evident, with no or mild MRC defects. Because all TRMT5 patients carry the p.Ile105Serfs*4 variant in one allele, the missense mutation in the trans allele (the reported p.Arg291His and p.Met386Val variants and p.Ile222Thr described in this work) might affect TRMT5 function distinctly, explaining the variability in MRC activity. Consequently, as very few families have been reported to date, further studies will be necessary to elucidate the effects of disrupting TRMT5 on MRC activity.

The three unrelated patients reported here shared a common phenotype and harboured the same c.312_315del (p.Ile105Serfs*4) mutation in the TRMT5 gene and a rare missense change c.665 T > C (p.Ile222Thr). The p.Ile105Serfs*4 frameshift change is relatively frequent in the healthy population, although it generates a premature stop codon and consequently a truncated protein that lacks the entire SAM-dependent methyltransferase domain. The rare c.665 T > C (p.Ile222Thr) missense change seems to be prevalent in our geographic region as it is carried by these three apparently unrelated families. Our structural analysis predicts that substitution of the hydrophobic Ile222 to a polar amino acid (Thr) most likely leads to the destabilisation of the D2 domain due to alterations of the hydrophobic interactions between some of its elements in a hydrophobic milieu. It is known that the tRNA methyltransferase activity of M. jannaschii aTrm5 is mainly accomplished by the D2-D3 domains, although Ile222 is located in the D2 domain, this residue appears to be outside the tRNA modification site. The D2 domain interacts with tRNA through phosphates and not nucleotide bases, indicating a non-specific structural interaction exists between D2 and tRNA. As described previously, the structure of the anticodon loop (position 32–38 of tRNA) in the complex of Trmt5 from M. jannaschii with tRNACys in the presence of SAM does not have the canonical conformation generally observed in tRNAs. The interaction of the D2 domain with tRNA is either forcing this non-canonical conformation of the anticodon loop or stabilising it. All these data suggest that a destabilisation of the D2 domain caused by the p.Ile222Thr mutation in our cases could affect tRNA binding and consequently, its modification.

In conclusion, TRMT5 mutations are responsible for a demyelinating sensorimotor neuropathy with congenital or infantile onset.

| Reference | Patient ID-sex | TRMT5 variants | RCC deficiency | Histochemical COX defect/acidosis | Age at onset (yr) | Clinical picture | NCS results | Neuroimaging findings |
|---|---|---|---|---|---|---|---|---|
| | | p.Ile105Serfs*4; Ile222Thr | | | | | | Scattered foci in the subcortical WM. |
| | | | | | | | | Muscle MRI; peroneal muscle fatty replacement, overall volume loss in thighs. |

Abbreviations: RCC, respiratory-chain complex; NCS, nerve conduction study; F, female; M, male; NA, not available; WM, white matter.
Screening for mutations in the TRMT5 gene should be considered when a patient is encountered with a global developmental delay, sensory predominant demyelinating neuropathy, pyramidal signs and mild cerebellar ataxia, even in the absence of a biochemical profile compatible with anOXPHOS deficiency. Ultrastructural muscle and nerve specimens might point to a mitochondrial aetiology when routine histopathological images appear normal. Given the absence of prominent structural and functional mitochondrial abnormalities, future cases are needed to confirm our findings.

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CONFLICT OF INTEREST
The authors report no conflict of interest.

ETHICS STATEMENT
The study was approved by the Hospital Universitari i Politècnic La Fe ethics committee, and written informed consent was obtained from the probands’ guardians.

AUTHOR CONTRIBUTIONS
HAE clinically characterised the three families, drafted the first manuscript and prepared the figures. NM and FME performed and supervised the brain and muscle MRIs. EMS performed and supervised the electrophysiological data analyses. JJV interpreted the neuromuscular pathological studies. RV and IA performed the muscle and the nerve pathology analyses. HAE, VL and CE conducted the genetic study on nuclear DNA. PSL performed the genetic study on mitochondrial DNA. CMM created the protein modelling. MAM analysed the activity of mitochondrial enzymes, PSL performed the genetic studies on mtDNA. MF, IP, MTV and JFVC provided clinical input and revised the manuscript for intellectual content. TS supervised the study and critically revised the manuscript.

DATA AVAILABILITY STATEMENT
The anonymised data used and analysed here will be shared on reasonable request.

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**SUPPORTING INFORMATION**

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