

TESIS DOCTORAL INTERNACIONAL

Caracterización fenotípica y molecular de las neuropatías hereditarias en la infancia y la adolescencia



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ABREVIATURAS

ACMG: colegio americano de genética médica y genómica, del inglés *American College of Medical Genetics and Genomics*

AD: autosómica dominante

ADM: *abductor digiti minimi*

ADN: ácido desoxirribonucleico

AH: *abductor hallucis*

AME: atrofia muscular espinal

APB: *abductor pollicis brevis*

AR: autosómica recesiva

ARNm: Ácido ribonucleico mensajero

ARNt: Ácido ribonucleico de transferencia

CMT: enfermedad de Charcot-Marie-Tooth

CMTNS: *CMT Neuropathy Score*

CMPedS: *CMT Pediatric Scale*

DAFOs: ortesis anti-equinas, del inglés *Device Ankle-Foot Orthosis*

DE: desviación estándar

EDB: *extensor digitorum brevis*

EMG: electromiograma de aguja

ENG: electroneurograma

HLF: Hospital Universitario y Politécnico La Fe

LMD: latencia motora distal

MLPA: *Multiplex Ligation-dependent Probe Amplification*

MRC: *Medical Research Council*

NGS: *Next Generation Sequencing*, en castellano conocido como 'secuenciación masiva' o de nueva generación

NHSM: neuropatías hereditarias sensitivas y motoras

NH: neuropatías hereditarias

NHMD: neuropatías hereditarias motoras distales

PAMC: Potencial de Acción Motor Compuesto

PANS: Potencial de Acción Nervioso Sensitivo

pb: pares de bases

PCR: Reacción en cadena de la polimerasa, del inglés *Polymerase Chain Reaction*

RCP: reflejo cutáneo plantar

RM: resonancia magnética

ROT: reflejo osteotendinoso

TA: *tibialis anterior*

VCM: velocidad de conducción nerviosa motora

VCN: velocidad de conducción nerviosa

WES: *Whole Exome Sequencing*

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0. RESUMEN

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EN CASTELLANO

ANTECEDENTES: La mayoría de las polineuropatías en la infancia son genéticamente determinadas, estimándose alrededor del 70-90% del total de neuropatías. Este grupo de neuropatías hereditarias (NH) comprende las NH tipo Charcot-Marie-Tooth y los síndromes hereditarios complejos bien sean enfermedades neurodegenerativas o errores innatos del metabolismo en los que la polineuropatía es una de sus características. La literatura médica es escasa en estudios de neuropatías hereditarias en población pediátrica, lo que puede ser atribuible a la escasez de programas nacionales e internacionales de bases de datos y escalas uniformes de evaluación pediátricas de aplicación generalizada.

OBJETIVOS: El objetivo principal es la caracterización genética y fenotípica de una serie de pacientes menores de 20 años con NH incluyendo la búsqueda de nuevos genes causantes. Los objetivos secundarios son: determinar la variabilidad de la gravedad de los distintos tipos de NH y estimar la sensibilidad de la escala CMTPedS como medida de progresión en las distintas NH.

METODOLOGÍA: Se trata de un estudio descriptivo longitudinal de 3 años de duración de una serie hospitalaria reclutados y valorados de forma prospectiva entre septiembre 2017 y septiembre de 2020 en la Unidad de Enfermedades Neuromusculares y Neuropediatría del Hospital Universitario y Politécnico La Fe (Valencia, España). Se incluyeron solo los pacientes que tenían menos de 20 años en el momento del inicio del estudio y contaban con un diagnóstico definitivo de neuropatía periférica de origen genético, aunque no se conociese el defecto genético causal tanto los casos índices como los secundarios (algún progenitor o hermano/a afectados). Los pacientes fueron estudiados desde el punto de vista clínico, neurofisiológico, de neuroimagen, de discapacidad (con la escala CMTPedS) y genético siguiendo los protocolos reflejados en la presente tesis.

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Para el estudio centrado en la utilidad de la escala CMTPedS en las formas motoras puras, se contó además con la aportación de pacientes por parte del grupo de investigación en neuropatías hereditarias en edades pediátricas de *The Children's Hospital at Westmead* (NSW, Australia). Los criterios de inclusión y exclusión que debían cumplir los pacientes procedentes de estos dos centros eran los mismos.

RESULTADOS: Se estudió un total de 110 pacientes con NH que en el momento del inicio tenían menos de 20 años. La mayoría pertenecen a la cohorte de pacientes remitidos al Hospital Universitario y Politécnico La Fe mientras que 8 de ellos fueron aportados por *The Children's Hospital at Westmead*. De los 102 pacientes procedentes del Hospital Universitario y Politécnico La Fe, tres presentaron un fenotipo tan único y compartían el mismo genotipo *TRMT5* que fueron descritos en un extenso trabajo aparte (Argente-Escrig et al., 2022). Así pues, 99 pacientes fueron los reflejados en el trabajo Argente-Escrig et al., 2021a. De estos 99 (59 hombres), 14 debutaron con neuropatía motora hereditaria distal (NHMd) y 85 debutaron con una forma sensitivomotora (NHSM) con 2/3 de subtipo desmielinizantes (Argente-Escrig et al., 2021a). El diagnóstico genético se logró en el 79,5% de las familias, con una tasa de detección de mutaciones en las formas desmielinizantes (88,7%) y axonales (89,5%), significativamente mayor que en las familias NHMd (18,2%). CMT1A fue el subtipo más frecuente (n = 37), seguido de los que tienen mutaciones heterocigóticas en los genes *GDAP1* (n = 9) o *GJB1* (n = 8). Se identificaron mutaciones en otros 15 genes, incluyendo una nueva variante patogénica en el gen *ATP1A1* (Argente-Escrig et al., 2021a).

La cohorte de 22 pacientes con NHMd (13 mujeres) de 19 familias procedió de los dos centros universitarios (Argente-Escrig et al., 2021b). 14 personas fueron sintomáticas en el primer año de vida. La discapacidad intelectual estuvo presente en 6 individuos y se observaron signos de motoneurona superior en 8. Se encontraron variantes patogénicas en 9 familias, más frecuentemente en

BICD2 (*BICD2-4*, *DYNC1H1-2*, *MFN2-2*, *GARS1-1*). Se identificó una nueva variante patogénica en el gen *GARS1* (Argente-Escrig et al., 2021b).

La CMTpedS detectó una progresión significativa de la enfermedad en todos los subtipos genéticos de NHSM (Argente-Escrig et al., 2021a), a un ritmo de 1,84 ($\pm 3,7$) durante 1 año ($p < 0,0005$, $n = 62$) y una tasa de 3,6 ($\pm 4,4$; $p < 0,0005$, $n = 45$) a los 2 años. También se detectó un empeoramiento significativo para CMT1A al año ($1,7 \pm 3,6$, $p < 0,05$) y a los 2 años ($4,2 \pm 4,3$, $p < 0,0005$). En las NHMd, la puntuación total de la CMTpedS a lo largo de 1 año se deterioró, en promedio, 1,5 puntos (DE 3,7) o 9% ($n = 12$), con variabilidad significativa en la tasa de progresión dentro de la cohorte (Argente-Escrig et al., 2021b).

Finalmente, se fenotipa en profundidad las neuropatías asociadas a dos genes distintos: *FGD4* y *TRMT5*. Los dos pacientes hermanos portadores de las variantes patogénicas c.514delG (p.Ala172Glnfs*28) y c.2211dupA (Ala738Serfs*5) en el gen *FGD4* debutaron en la adolescencia y mostraron un fenotipo muy leve a diferencia de lo publicado previamente (Argente-Escrig et al., 2019). La proteína truncada p.Ala738Serfs*5 puede haber conservado parcialmente la actividad de FGD4 ya que se conservan los principales dominios funcionales. Las mutaciones recesivas en el gen *TRMT5* en tres pacientes procedentes de tres familias distintas se asociaron con un fenotipo no descrito previamente (Argente-Escrig et al., 2022). Se presentaron con retraso global del desarrollo, neuropatía desmielinizante de predominio sensitivo de inicio congénito o infantil, signos piramidales, ataxia cerebelosa leve y ausencia de un perfil bioquímico compatible con una deficiencia de OXPHOS. El análisis patológico rutinaria de músculo y nervio en estos pacientes resultó aparentemente normal mientras que el estudio ultraestructural estaba claramente alterado.

CONCLUSIONES: Nuestra serie pediátrica presenta características diferenciadoras con otras cohortes descritas por la mayor proporción de portadores heterocigotos en el gen *GDAP1*. La afectación del tracto piramidal y

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la cognitiva son frecuentes en las formas motoras puras en la edad pediátrica. Se confirmó la progresión de las NHSM a los dos años de seguimiento medida con la CMTPedS, y como novedad se detectó que la CMTPedS es sensible al cambio también en el primer año de seguimiento de las NHSM. Los presentes datos apoyan a la escala CMTPedS como una medida de discapacidad sensible a la progresión al año también en las formas motoras y nuestro estudio apunta a que la CMTpedS se puede adaptar a los pacientes con formas motoras. El fenotipado en profundidad y el exhaustivo análisis genético realizado nos ayudan a comprender los mecanismos patogénicos asociados a las diferentes variantes y su influencia en el fenotipo final. Las muestras ultraestructurales de músculos y nervios pueden indicar una etiología mitocondrial en casos en que las imágenes histopatológicas de rutina parecen normales.

EN INGLÉS

BACKGROUND: Most polyneuropathies in childhood are genetically determined, with an estimated 70-90% of all neuropathies. Inherited peripheral neuropathies (IPN) include Charcot-Marie-Tooth disease and complex hereditary syndromes, whether they are neurodegenerative diseases or inborn errors of metabolism in which polyneuropathy is one of their characteristics. Medical literature is scarce on studies of IPN in the pediatric population, which may be attributable to the short supply of national and international database programs and uniform pediatric evaluation scales for general application.

OBJECTIVES: The main objective is the genetic and phenotypic characterization of a series of IPN patients under 20 years of age, including the search for new causative genes. The secondary objectives are the following: to determine the variability of the severity of the different types of IPN and to estimate the sensitivity of the CMTPedS scale as a measure of progression in the different IPN.

METHODS: This is a 3-year longitudinal descriptive study of a hospital-based series prospectively recruited and assessed between September 2017 and September 2020 in the Neuromuscular Diseases and Child Neurology Unit of the Hospital Universitario y Politécnico La Fe (Valencia, Spain). Only patients who were under 20 years of age at the start of the study and had a definitive diagnosis of peripheral neuropathy of genetic origin were included, even if the causal genetic defect was not known, both in index cases and in secondary cases (either parent or affected sibling). Patients were studied from the clinical, neurophysiological, neuroimaging, disability (with the CMTPedS scale) and genetic perspective following the protocols reflected in this thesis.

The research group on inherited neuropathies at *The Children's Hospital at Westmead* (NSW, Australia) contributed with patients with pure motor IPN for the study on the utility of the CMTPedS scale in pure motor forms. The inclusion and exclusion criteria that patients from these two centers had to meet were the same.

RESULTS: A total of 110 patients with IPN who were under 20 years old at onset were studied. Most belonged to the cohort of patients referred to Hospital Universitario y Politécnico La Fe, while 8 of them were contributed by The Children's Hospital at Westmead. Of the 102 patients from the Hospital Universitario y Politécnico La Fe, three presented such a unique phenotype and shared the same *TRMT5* genotype that they were described in an extensive separate study (Argente-Escrig et al., 2022). Thus, 99 patients were reflected in the study Argente-Escrig et al., 2021a. Of these 99 (59 male), 14 presented with distal hereditary motor neuropathy (dHMN) and 85 had a sensorimotor form with 2/3 of the demyelinating subtype (Argente-Escrig et al., 2021a). Genetic diagnosis was achieved in 79.5% of families, with a detection rate of mutations in demyelinating forms (88.7%) and axonal forms (89.5%), significantly higher than in dHMN families (18.2%). CMT1A was the most frequent subtype (n = 37), followed by those with heterozygous mutations in the *GDAP1* (n = 9) or *GJB1* (n

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= 8) genes. Mutations in another 15 genes were identified, including a new pathogenic variant in the *ATP1A1* gene (Argente-Escrig et al., 2021a).

The cohort of 22 dHMN patients (13 female) from 19 families came from the two teaching hospitals (Argente-Escrig et al., 2021b). 14 people were symptomatic in the first year of life. Intellectual disability was present in 6 individuals and upper motor neuron signs were seen in 8. Pathogenic variants were found in 9 families, most frequently in *BICD2* (*BICD2-4*, *DYNC1H1-2*, *MFN2-2*, *GARS1-1*). A new pathogenic variant in the *GARS1* gene was identified (Argente-Escrig et al., 2021b).

The CMTpedS detected significant disease progression in all sensorimotor genetic subtypes (Argente-Escrig et al., 2021a), at a rate of 1.84 (± 3.7) over 1 year ($p < 0.0005$, $n = 62$) and a rate of 3.6 (± 4.4 ; $p < 0.0005$, $n = 45$) at 2 years. Significant worsening was also detected for CMT1A at 1 year (1.7 ± 3.6 , $p < 0.05$) and at 2 years (4.2 ± 4.3 , $p < 0.0005$). In the dHMN, the CMTpedS total score over 1 year deteriorated, on average, 1.5 points (SD 3.7) or 9% ($n = 12$), with significant variability in the rate of progression within 1 year of follow-up (Argente-Escrig et al., 2021b).

Finally, neuropathies associated with two different genes are phenotyped in depth: *FGD4* and *TRMT5*. The two sibling patients who were carriers of the pathogenic variants c.514delG (p.Ala172Glnfs*28) and c.2211dupA (Ala738Serfs*5) in the *FGD4* gene had their onset in adolescence and showed a very mild phenotype in contrast to what was previously published (Argente - Escrig et al., 2019). The p.Ala738Serfs*5 truncated protein may have partially conserved FGD4 activity since the main functional domains are preserved. Recessive mutations in the *TRMT5* gene in three patients from three different families were associated with a phenotype not previously described (Argente-Escrig et al., 2022). They presented with global developmental delay, predominantly sensory demyelinating neuropathy of congenital or infantile onset, pyramidal signs, mild cerebellar ataxia, and no biochemical profile consistent with

OXPPOS deficiency. The routine pathological analysis of muscle and nerve in these patients was apparently normal while the ultrastructural study was clearly abnormal.

CONCLUSIONS: Our pediatric series presents differentiating characteristics with other cohorts by the higher proportion of heterozygous carriers in the *GDAP1* gene. Pyramidal tract and cognitive involvement are frequent in the pure motor forms in the pediatric population. The progression of the sensorimotor forms was confirmed at two years of follow-up measured with the CMTPedS, and as a novelty it was detected that the CMTPedS is sensitive to change also in the first year of follow-up. The present data support the CMTPedS scale as a measure of disability sensitive to progression at one year also in motor forms, and our study suggests that a scale modelled on CMTPedS might be useful for pediatric patients with motor forms. The in-depth phenotyping and exhaustive genetic analysis carried out help us to understand the pathogenic mechanisms associated with the different variants and their influence on the final phenotype. Ultrastructural muscle and nerve specimens may point to a mitochondrial etiology in cases where routine histopathologic imaging appears normal.

RESUMEN

1. INTRODUCCIÓN

INTRODUCCIÓN

A. GENERALIDADES DE LAS NEUROPATÍAS HEREDITARIAS EN EDAD PEDIÁTRICA

CONCEPTO CLÍNICO Y ABORDAJE DIAGNÓSTICO

Las neuropatías hereditarias (NH) son enfermedades clínica y genéticamente heterogéneas causadas por la degeneración lentamente progresiva y crónica del sistema nervioso periférico. En líneas generales, la clínica se caracteriza por dificultad para andar y deformidad de pies y, con el progreso de la enfermedad, se va afectando la musculatura de la pierna, muslo y manos (Pareyson y Marchesi, 2009).

Las NH constituyen el 70-90% de las neuropatías en la infancia (Jani-Acsadi et al., 2015; Shabo et al., 2007) pero son pocos los estudios existentes sobre éstas. Las NH en la infancia comparten el mismo abordaje conceptual que en los adultos. No obstante, existen unas características diferenciales: una mayor variedad de presentaciones clínicas, dificultades en un minucioso examen neurológico y neurofisiológico en los pacientes más jóvenes, la existencia de enfermedades metabólicas raras con presentación polineuropática ocasional y una gran variedad de genes causantes. Así pues, el paso más importante en el abordaje diagnóstico es siempre el examen clínico el cual debe ir más allá de los hallazgos neuromusculares y se debe repetir a lo largo de la evolución.

Los parámetros principales implicados en la evaluación clínica, así como en la caracterización de una neuropatía con sospecha de origen hereditario son:

- carácter puro vs complejo de la neuropatía
- tipo de nervio periférico implicado
- fisiopatología de la degeneración de la fibra nerviosa
- patrón de herencia aparente
- periodo vital

INTRODUCCIÓN

a) Neuropatías ‘puras’ vs ‘complejas’

Las neuropatías puras son aquellas que afectan exclusivamente al sistema nervioso periférico. Suelen presentar un curso lentamente progresivo, aunque existe una gran heterogeneidad clínica y genética, en la actualidad se conocen > 90 genes responsables (<http://neuromuscular.wustl.edu/time/hmsn.html>).

Por lo que respecta a las neuropatías complejas (también conocidas como neuropatías plus), éstas pueden ocurrir como complicación tardía de una enfermedad genética crónica, como un elemento principal de una enfermedad pleiotrópica o en el contexto de un síndrome congénito multisistémico (Shabo et al., 2007). Dentro de éstas puede ocurrir:

- 1) Que el síndrome esté confinado al *sistema nervioso* incluyendo tanto el periférico como el central. Éste es el caso de las paraparesias espásticas hereditarias y las ataxias espinocerebelosas con neuropatía.
- 2) Que se trate de enfermedades *multisistémicas* y se afecte tanto el sistema nervioso periférico como otros sistemas no neurológicos (cardíaco, renal, visual, etc.). Este grupo engloba a las enfermedades metabólicas entre las que encontramos las debidas a defectos lisosomales, peroxisomales, mitocondriales, del metabolismo de los aminoácidos, de la glicosilación post-transcripcional y de los transportadores sistémicos específicos (Landrieu y Baets, 2013).

b) Tipo de fibra nerviosa implicada

De acuerdo con la clínica y los resultados del electroneurograma (ENG) las NH se clasifican en tres grandes grupos (Dyck, 1984). El grupo principal y el más prevalente es el de las neuropatías hereditarias sensitivas y motoras (NHSM), también conocidas como enfermedad de Charcot-Marie-Tooth (CMT). La atrofia

muscular espinal (AME) y las neuropatías hereditarias motoras distales (NHMd) se caracterizan por una afectación selectiva de las neuronas motoras periféricas de una forma no longitud-dependiente (neuronopatía) o longitud-dependiente (neuropatía), respectivamente. Las neuropatías que afectan selectivamente a las neuronas sensitivas +/- autonómicas, conocidas como neuropatías hereditarias sensitivas y autonómicas, son raras en la edad pediátrica.

Esta subdivisión en función del tipo de nervio periférico afectado es sobre todo a modo de enfoque práctico, pero existe un solapamiento entre los diferentes fenotipos.

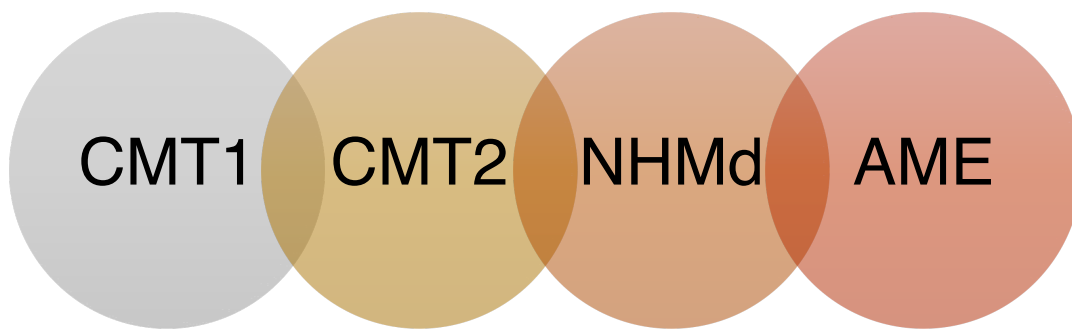


Figura 1. Continuum en las neuropatías periféricas hereditarias.

En ocasiones, especialmente en individuos muy jóvenes, el único modo de establecer la implicación de las neuronas sensitivas es con los estudios de ENG dado que los signos sensitivos suelen ser dudosos en la exploración clínica. Los siguientes motivos convierten la realización de estudios neurofisiológicos en la población pediátrica en un reto. Los niños, por un lado, presentan una tolerancia baja por lo que el ENG y electromiograma de aguja (EMG) se limita normalmente a medidas básicas. Por otro, la interpretación de los resultados del ENG y EMG es difícil puesto que en los cinco primeros años de vida los nervios periféricos aún están en proceso de maduración y los valores normales son variables (Parano et al., 1993; García et al., 2000).

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c) Fisiopatología de la degeneración de la fibra nerviosa

Cuando los estudios de biopsia de nervio y los electrofisiológicos se convirtieron en rutina en la práctica clínica en el adulto, sus resultados mostraron unos patrones diferenciales coherentes: el desmielinizante y el axonal. La patología de las células de Schwann conduce a una desmielinización segmentaria y da lugar a un enlentecimiento marcado de la velocidad de conducción nerviosa (VCN). Mientras que la degeneración puramente axonal, cuando afecta a una proporción suficiente de fibras nerviosas mielínicas gruesas (tipo $A\alpha$), da lugar a una VCN casi normal. Así pues, en función de la VCN motora (VCM) de un nervio de la extremidad superior las NHSM se dividen clásicamente en dos categorías: desmielinizante o CMT1 si < 38 m/s y axonal o CMT2 si > 38 m/s (Harding y Thomas, 1980). La categoría de CMT 'intermedio' (CMT-I), considerada aquella con valores entre 25 y 45 m/s y hallazgos patológicos variables (Davis et al, 1978), presenta unos criterios de difícil delimitación (Berciano et al., 2017).

No obstante, la correlación de los valores de la VCN con el mecanismo patológico subyacente es sólo útil parcialmente en el caso de las NH de la infancia por diversas razones. En primer lugar, es difícil distinguir entre el subtipo axonal del desmielinizante cuando los potenciales de acción no se pueden evocar o están muy disminuidos a la estimulación. En segundo lugar, un desarrollo anormal puede afectar a la desmielinización sin que se evidencie la desmielinización segmentaria, al crecimiento axonal sin evidenciarse la degeneración retrógrada, o ambos procesos simultáneamente, haciendo que los valores de VCN sean más difíciles de correlacionar con los hallazgos neuropatológicos (Berciano & Combarros, 2003).

d) Patrón de herencia

El modo de herencia en la familia es un elemento importante para la subclasificación de las NH las cuales se transmiten como enfermedades monogénicas con alta penetrancia. Se han descrito herencias autosómica dominante (AD), ligadas al X y autosómica recesivas (AR). Especialmente en las neuropatías graves de inicio temprano, hay una proporción sustancial de

mutaciones dominantes *de novo* que aparecen como casos esporádicos. En familias pequeñas es difícil de distinguir estos casos de las formas recesivas. Además, aunque la mayoría de defectos genéticos se comportan como dominantes o como recesivos, unas cuantas excepciones pueden presentar ambos patrones de herencia (Cuesta et al., 2002; Zimón et al., 2011).

e) Periodo vital

Esta categoría clínica le es familiar a los pediatras dado que cada periodo de la infancia y la niñez presenta un diagnóstico diferencial distinto. En el caso de las neuropatías periféricas, la categoría pediátrica más relevante es el de las 'neuropatías congénitas' que son aquellas que aparecen en el periodo perinatal, en el neonato o en la infancia precoz. Muchas neuropatías congénitas son sindrómicas e incluyen anomalías del desarrollo muy específicas de la práctica pediátrica y están asociadas a defectos genéticos distintos de aquellas que se encuentran en las neuropatías primarias. Los neonatos muestran una clínica grave consistente en hipotonía, arreflexia, alteraciones del llanto y la succión, e insuficiencia respiratoria (Yiu y Ryan, 2012a; Sevilla et al., 2011). Cuando debuta en la infancia precoz aparece un retraso del inicio de la marcha de más de 18 meses, deformidad de pies, y pérdida de la sensibilidad (Yiu y Ryan, 2012b; Baets et al., 2011). Hay que tener en cuenta que las AME cursan con hipotonía en el neonato/infancia precoz. La base genética más frecuentes del AME es las mutaciones bialélicas del gen *SMN1* dando lugar al subtipo más prevalente conocido como AME 5q (Prior et al., 2016). Sin embargo, las AME no debidas a mutaciones del gen *SMN1* se presentan con debilidad de predominio distal por lo que la clínica se solapa con las neuropatías hereditarias motoras (Darras, 2015). Es por ello que las AME no debidas a mutaciones del gen *SMN1* (o AME no5q) también serán incluidas en la presente tesis.

Cuando las NHs debutan en la infancia tardía o en la juventud no hay retraso del inicio de la marcha y se pueden presentar con caídas frecuentes, dificultad para andar de talones (figura 2), hipo o arreflexia y deformidad de pies (pies cavos,

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dedos en martillo). A medida que la enfermedad avanza se va afectando la musculatura de la pierna, muslo y manos confiriendo el típico aspecto de “patas de cigüeña (figura 3A) y manos “en garra” (figura 3B). Estas formas tardías son más frecuentes y se parecen al “fenotipo clásico” del adulto (Pareyson y Marchesi, 2009).

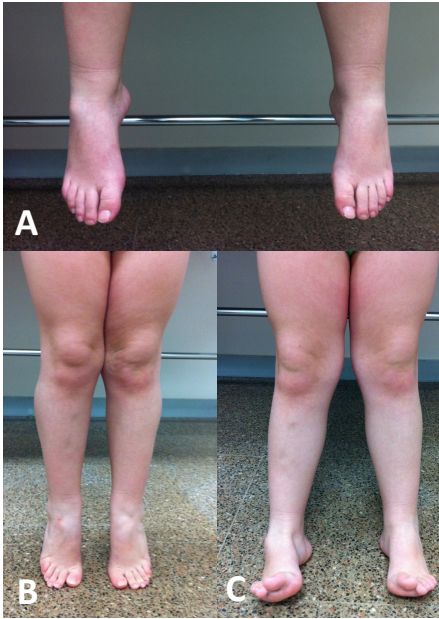


Figura 2. Niña con fenotipo leve de NH. Se muestra la actitud caída de los pies (A) y la capacidad para ponerse de puntillas (B) pero no de talones (C).

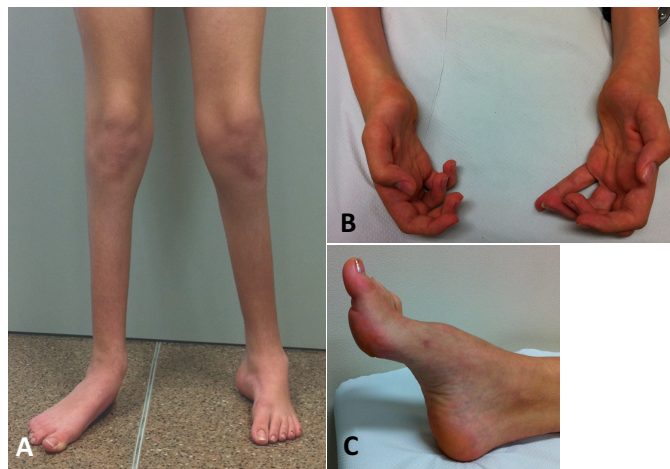


Figura 3. Adolescente de 14 años con fenotipo grave de NH. Se aprecia la atrofia de piernas en “pata de cigüeña” (A), las manos “en garra” (B) y los pies cavos con dedos en martillo (C).

BASES GENÉTICAS Y CLASIFICACIÓN

La primera causa genética descrita de CMT fue la duplicación en tándem del cromosoma 17p11.2-12, que contiene el gen PMP22, causante de la forma CMT1A la cual es el subtipo más prevalente (50% de los casos) (Sivera et al., 2013; Murphy et al., 2012; Saporta et al., 2011). Aunque el patrón clínico es

heterogéneo, la genética lo es mucho más y el descubrimiento de genes responsables de las mismas ha sido exponencial desde la disponibilidad de la tecnología de secuenciación masiva (NGS, del nombre en inglés *Next Generation Sequencing*). En la actualidad se conocen > 90 genes responsables de NH primarias (<http://neuromuscular.wustl.edu/time/hmsn.html>).

Abordar el diagnóstico genético en las formas no CMT1A es muy laborioso y costoso debido a la gran heterogeneidad genética. Empleando la secuenciación tipo Sanger, la caracterización genética en pacientes adultos con CMT osciló entre en el 66% (Murphy et al., 2012; Saporta et al., 2011) y el 83,3% de la serie de la Unidad de Neuromuscular del HUPLF (Sivera et al., 2013). En la serie pediátrica más larga se caracterizaron el 45% estudiando 11 genes (Baets et al., 2011).

Como apuntamos anteriormente, en la actualidad se emplea una clasificación que combina la neurofisiología, la clínica, las alteraciones genéticas y el patrón de herencia (Payreson y Marchesi, 2009; Reilly et al., 2011). Para identificar las causas genéticas específicas dentro de los grandes grupos (desmielinizantes u axonales) se sigue de una letra en mayúscula p.e. CMT1A, CMT2A, etc (Payreson y Marchesi, 2009; Reilly et al, 2011). Para mayor claridad se nombra la neuropatía por el gen causante y entre paréntesis se añade la letra correspondiente al subtipo. A continuación, se describen los genotipos más frecuentes en función de los principales tipos de NHSM o CMT y los más relevantes para la presente tesis (por debutar en la infancia o por haber sido identificado en nuestra cohorte).

CMT desmielinizante autosómico dominante (CMT1 AD).

Se trata de la forma más frecuente de las CMT en las series pediátricas (Fernández-Ramos et al, 2015; Cornett et al, 2016). Se subclasifica en:

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Duplicación PMP22 (CMT1A). En la infancia y adolescencia suele suponer el 50% de los casos (Wilmshurst y Ouvrier, 2011; Cornett et al, 2016) por lo que se convierte en la causa más frecuente de CMT en edad pediátrica. Es provocada por una duplicación en 17p12, que contiene el gen *PMP22*, el cual es sobreexpresado (Jani-Acsadi et al, 2015; Reilly et al, 2011). Suele desarrollar el ‘fenotipo clásico’, aunque su presentación puede ser variable e incluso pueden desarrollar importantes malformaciones esqueléticas y debilidad proximal (Berciano et al, 2003; Pareyson y Marchesi, 2009).

Hay que diferenciar el CMT1A de la Neuropatía Hereditaria con susceptibilidad a las Parálisis por Presión que también es AD pero la base molecular es la delección del segmento 17p12 (Chance et al, 1993). Estos pacientes presentan episodios recurrentes y transitorios de debilidad focal o pérdida sensitiva generalmente precipitados por presión en la distribución de nervios individuales o plexos. Puede estar asociada a hiporreflexia y pies cavos. Lo característico en la anatomía patológica es la presencia de “tomácula” (excesivos pliegues de mielina alrededor del axón). Raramente se manifiesta en niños (Felice et al, 1999).

MPZ (CMT1B). Está causada por mutaciones en el gen *MPZ (Myelin Protein Zero)* y puede dar lugar a varios fenotipos. Uno semejante a CMT1A con VCM muy lenta (menores de 10 m/s), de inicio congénito (neuropatía hipomielinizante congénita) en la que la mayoría de los axones están hipomielinizados o carentes de mielina en la biopsia sural (Sevilla et al, 2011; Sanmaneechai et al, 2015). Otro fenotipo de inicio tardío con VCM en rango intermedio o axonal (De Jonghe et al, 1999) que cada vez está siendo más relevante como causa de NH de inicio tardío (Callegari et al, 2019).

LITAF (CMT1C). Mutaciones en el gen *LITAF* (*Lipopolysaccharide-Induced Tumor Necrosis Factor*) provocan una sintomatología similar a la de los CMT1A (Jani-Acsadi et al, 2015).

EGR2 (CMT1D). Las neuropatías por mutaciones en *EGR2* (*Early Growth Response 2*) cursan un rango de fenotipos que van desde las formas hipomielinizantes congénitas (Warner et al, 1998; Lupo et al, 2020), neuropatías desmielinizantes más o menos graves de inicio en la infancia (Warner et al, 1998) y formas axonales leves o moderadas de inicio en el adulto (Sevilla et al 2015). Todas las mutaciones descritas, excepto dos que segregan autosómico recesivas (CMT4E), segregan autosómico dominante o se presentan como mutaciones *de novo* dominantes en casos esporádicos.

NEFL (CMT1F). Causada por mutaciones en el gen *NEFL* (*Neurofilament Protein, Light Polypeptide*) provoca formas graves y de inicio temprano (Jordanova et al, 2003). Son poco frecuentes y existen formas recesivas con temblores y ataxia cerebelosa (Abe et al, 2009). El subtipo CMT1D y CMT1F constituyen el 2% de los CMT en edad pediátrica (Yiu y Ryan, 2012b).

CMT desmielinizante autosómica recesiva (CMT1 AR o CMT4).

Es menos frecuente que CMT1, pero su proporción es relativamente alta en comunidades consanguíneas (Sivera et al, 2013). Estos pacientes presentan un inicio más precoz y una forma más grave con pérdida temprana de la deambulación.

GDAP1 (CMT4A). Causada por mutaciones del gen *GDAP1* (*Ganglioside-induced Differentiation-Associated Protein 1*). Las mutaciones en *GDAP1* se describieron en pacientes con herencia AR, con fenotipo axonal y desmielinizante (Cuesta et al 2002, Baxter et al 2002). Posteriormente se han

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hallado mutaciones que segregan de forma AD y presentan un fenotipo más leve (Zimón et al, 2011). Las formas dominantes y axonales (CMT2K) son frecuentes en toda España (Sivera et al, 2017) pero lo son especialmente en la Comunidad Valenciana por el CMT asociado a la mutación p.Arg120Trp (Sivera et al, 2010). Las formas recesivas tienen un inicio anterior a los dos años que progresa rápidamente a atrofia distal. Algunos necesitan el uso de silla de ruedas antes de los 20 años. El cuadro se puede acompañar de paresia de las cuerdas vocales y alteraciones diafragmáticas (Cuesta et al, 2002; Sevilla et al, 2008).

MTMR2 (CMT4B1). Está causada por mutaciones en el gen *MTMR2 (Myotubularin Related Protein 2)*, cursa con afectación de nervios craneales e inicio en la infancia y presentan mielina doblada a nivel focal en las biopsias de nervio sural (Bolino et al, 2000).

SBF2 (CMT4B2). Esta causada por mutaciones en el gen *SBF2 (Set-Binding Factor 2)* y cursa con glaucoma (Senderek et al, 2003).

SH3TC2 (CMT4C). Es debida a mutaciones en el gen *SH3TC2 (SH3 domain and tetratricopeptide repeat domain 2)*. Se está convirtiendo en la forma más común de CMT1 AR y la más frecuente en España (Sivera et al, 2013). La mayoría de los niños tienen un inicio de la marcha entre los 24-30 meses (Yiu y Ryan, 2012b). En las biopsias de los nervios podemos observar bulbos en capas de cebolla en la membrana basal (Gebreëls-Festen et al, 1999). En nuestro país la mayoría de los casos con mutaciones de *SH3TC2* se da en pacientes de etnia gitana, habiendo 2 mutaciones fundadoras privativas (Claramunt et al 2007).

Existen tres formas de CMT4 que están limitadas a pacientes de procedencia romaní:

- NDRG1 (CMT4D). Se encuentra asociada a mutaciones en el gen *NDRG1 (N-myc downstream regulated gene 1)* y ocasiona sordera, escoliosis, ataxia,

deformidades en los pies y, a veces, atrofia proximal (Kalaydjieva et al, 2000).

- CTDP1 (CCFDN - Catarata Congénita, Dismorfismo Facial y síndrome Neuropático). En relación con mutaciones de *CTDP1 (Carboxy-Terminal Domain Phosphatase 1)* y presenta desarrollo de cataratas congénitas dismorfismo facial, retraso de crecimiento, hipogonadismo y movimientos extrapiramidales (Tounev et al, 1999; Walter et al, 2014).
- HK1 (CMT4G o tipo Russe). Causado por mutaciones en el gen *HK1 (Hexokinase-1)*. Representa una forma grave descrita extensamente en un estudio español (Sevilla et al, 2013).

En España las mutaciones más frecuentes en población romaní por orden de frecuencia son: *SH3TC2*, *HK1* y *NDRG1* (Sevilla et al, 2013).

PRX (CMT4F). Asociada a mutaciones en el gen *PRX (Periaxin)* y presentan un CMT de inicio temprano con predominio de afectación sensitiva con ataxia, neuralgia trigeminal, escoliosis y deformidades en los pies (Guilbot et al, 2001).

FGD4 (CMT4H). Es debida a mutaciones del gen *FGD4 (FYVE, RhoGEF And PH Domain-containing protein 4)* (Delague et al, 2007). Se inicia en la infancia temprana y la progresión es lenta con escoliosis, ataxia sensorial, deformidades en los pies, siringomielia espinal, debilidad distal y mielina no doblada en la biopsia (Stendel et al, 2007; Fabrizi et al, 2009). Desde que se describieron las dos primeras familias con CMT4H (Delague et al, 2007), ésta se ha considerado como una enfermedad desmielinizante de aparición muy temprana con un fenotipo grave con pérdida temprana de la deambulación. Sin embargo, conforme ha aumentado el número de familias CMT4H se han ido detectando formas más leves. Aún siendo leves, una característica común a todos fue el inicio en la infancia. De hecho, todos los pacientes comenzaron con síntomas antes de cumplir los 9 años (Houlden et al, 2009; Fabrizi et al, 2009; Baudot et al, 2012; Arai et al, 2013; Boubaker et al, 2013; Sivera et al, 2013; Zimón et al, 2015; Hyun et al, 2015; Stendel et al, 2015; Zis et al, 2017; Kondo et al, 2017).

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FIG4 (CMT4J). Causada por mutaciones en el gen *FIG4* (*FIG4 Phosphoinositide 5-Phosphatase*) que se presenta con súbita debilidad asimétrica distal y proximal grave (Zhang et al 2008; Nicholson et al, 2011). Aspectos que destacar de este subtipo de CMT son la gran variabilidad en el inicio (desde la infancia a la edad adulta) y la presencia de características propios de las neuropatías adquiridas como son los bloqueos de conducción y la desmielinización no uniforme (Hu et al, 2018).

SURF1 (CMT4K). Los pacientes portadores de mutaciones en el gen *SURF1* (*surfeit 1*) generalmente muestran ataxia de la marcha, retraso en el crecimiento, regresión, acidemia láctica y neuropatía sensitivomotora, ya sea axonal o desmielinizante (síndrome de Leigh) (Wedatilake et al, 2013). Sin embargo, se han descrito dos familias con neuropatía grave de inicio en la infancia con acidemia láctica (Echaniz-Laguna et al, 2013).

Dentro de las neuropatías **desmielinizantes autosómico recesivas de fenotipo complejo**, encontramos las siguientes de interés para esta tesis:

- ARSA (LDM - Leucodistrofia metacromática). Las leucodistrofias metacromáticas se deben a mutación homocigota o heterocigota compuesta en el gen *ARSA* (Arilsulfatasa A) y comprenden varios trastornos alélicos (Kihara et al., 1982): formas infantiles tardías, juveniles y adultas, deficiencia parcial de sulfato de cerebrósido y deficiencia de pseudoarilsulfatasa A. La forma de inicio infantil tardía debuta antes de los 30 meses de edad. Los hallazgos de presentación típicos incluyen debilidad, hipotonía, torpeza, caídas frecuentes, caminar de puntillas y disartria. A medida que la enfermedad progresa, el lenguaje, las habilidades cognitivas y motoras gruesas y finas regresan. En las etapas finales, los niños tienen espasmos tónicos, posturas de descerebración y desconocimiento general de su entorno. La afectación de SNC (con

alteraciones en sustancia blanca cerebral) suele ser previa a la aparición de la neuropatía desmielinizante (Kehrer et al, 2011).

- *TRMT5* (COXPD26 - Deficiencia combinada de fosforilación oxidativa 26)

En tres familias hasta la fecha, las mutaciones en *TRMT5* (ARNt metiltransferasa 5) se han asociado con intolerancia al ejercicio, neuropatía escasamente caracterizada, espasticidad, retraso en el desarrollo y deficiencia mitocondrial en la actividad del complejo I y IV de la cadena respiratoria en el músculo esquelético (Powel et al, 2015; Tarnopolsky et al, 2017).

CMT axonal autosómica dominante (CMT2 AD).

Las formas axonales son menos frecuentes que las CMT1 (Saporta et al, 2011). Si lo comparamos con la CMT1 AD, en la CMT2 AD el inicio suele ser posterior y las deformidades menos frecuentes y graves (Jani-Acsadi et al, 2015). A continuación, se exponen los principales subtipos de inicio en la infancia y adolescencia.

MFN2 (CMT2A). Causada por mutaciones del gen *MFN2* (*Mitofusin 2*). Suele suponer el 6% de las formas pediátricas (Cornett et al, 2016). Aparecen dos presentaciones: una de inicio temprano sobre los 5 años, con debilidad distal y problemas en la marcha que van progresando hasta necesitar el uso de silla de ruedas, acompañándose de atrofia (Nicholson et al, 2008); y una de inicio tardío grave (Verhoeven et al, 2006). Es característica la asociación con atrofia óptica (CMT6A) (Züchner et al, 2006). Se han descrito alteraciones en sustancia blanca en la RM cerebral (Verhoeven et al, 2006). Estudios recientes de historia natural mejorarán el diseño de ensayos clínicos de futuras terapias racionales (Pipis et al, 2020).

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RAB7 (CMT2B). Causada por mutaciones en el gen *RAB7 (Ras-associated protein Rab-7)*. Cursa con hipoestesia distal, hiperqueratosis e incluso fenotipos ulcero-mutilantes (Houlden et al, 2004).

TRPV4 (CMT2C). Debida a mutaciones en el gen *TRPV4 (Transient Receptor Potencial Cation Channel, subfamily V, member 4)* y causa diversas variedades alélicas (Auer-Grumbach et al, 2010). CMT2C cursa con parálisis de las cuerdas vocales, del diafragma, los músculos intercostales y los músculos proximales (Chen et al, 2010). Además, las variantes patogénicas de *TRPV4* pueden causar otras dos neuropatías hereditarias de predominio motor: la atrofia muscular escapuloperoneal (Deng et al, 2010) y la atrofia muscular congénita espinal distal (Astrea et al, 2010).

GARS1 (CMT2D). Es causada por la mutación del gen *GARS1 (Glycyl-TRNA Synthetase 1)*. Estos pacientes desarrollan atrofia y debilidad de la musculatura intrínseca de la mano (con calambres y dolor), afectando después a la musculatura distal de los miembros inferiores (Antonellis et al, 2003). Puede afectar gravemente a niños y presentarse como una forma grave infantil puramente motora tipo AME (James et al, 2006).

NEFL (CMT2E). Provocada por la mutación en el gen *NEFL (Neurofilament protein, light chain)*. Se asocia a ataxia, deformidades en los pies, alteración en la sensibilidad profunda y superficial e inicio en la primera década. Suele tener niveles de CPK elevados (Mersiyanova et al, 2000).

MPZ (CMT2J). Asociada a mutaciones en el gen *MPZ* (Chapon et al, 1999). De esta forma axonal es característico el inicio tardío, la afectación pupilar y la hipoacusia (Kabzińska et al, 2007).

MORC2 (CMT2Z). De reciente descripción, debida a mutación en el gen *MORC2 (Microrchidia Family CW-Type Zinc Finger 2)*. Su presentación clínica es variable, desde formas de inicio temprano con retraso en el crecimiento y

dismorfia craneofacial (Guillen Sacoto et al, 2020) a formas de inicio en infancia o adolescencia que empiezan con calambres, alteración sensitiva y debilidad distal, que llega a progresar hasta nivel proximal (Sevilla et al, 2016).

ATP1A1 (CMT2DD). Se trata de neuropatía sensitivomotora axonal que afecta principalmente a las extremidades inferiores y está causado por mutaciones heterocigotas en *ATP1A1*, que codifica la subunidad alfa-1 del Na²⁺/K⁺-ATPasa (Lassuthova et al, 2018). La edad de inicio y la gravedad del trastorno son muy variables, incluso dentro de las familias. Los pacientes siguen siendo ambulatorios incluso en etapas avanzadas de la enfermedad, aunque algunos pueden requerir dispositivos ortopédicos (Lassuthova et al, 2018). Posteriormente, se identificaron mutaciones heterocigóticas *de novo* en el gen *ATP1A1* en individuos con hipomagnesemia renal, crisis epilépticas refractarias y discapacidad intelectual (Schlingmann et al, 2018) y en un niño con paraplejía espástica y discapacidad intelectual con estudio de conducciones nerviosas normal (Stregapede et al, 2020).

CMT axonal autosómica recesiva (CMT2 AR).

Es muy rara comparada con la CMT2 AD.

LMNA (CMT2B1). Causada por la mutación del gen *LMNA (Lamina A/C)*. Se suele presentar en la segunda década con un fenotipo CMT grave y afectación proximal (De Sandre-Giovannoli et al, 2002). También se asocia a ciertas distrofias musculares tipo Emery-Dreifuss (Raffaele Di Barletta et al, 2000).

GDAP1 (CMT2H/K). Entidad descrita anteriormente en CMT4A. Es la más frecuente de las formas recesivas y hay que pensar en ella siempre que nos encontremos ante un fenotipo CMT grave de inicio temprano (Sivera et al, 2017).

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CMT ligada al cromosoma X (CMTX).

GJB1 (CMTX1). Es secundario a mutaciones en el gen *GJB1 (Gap Junction Protein Beta-1)*. Los hombres suelen estar afectados más gravemente, con un inicio en primera o segunda década, debilidad en piernas y cierta asimetría (Birouk et al, 1998). A veces hay hiperreflexia, dolor y piramidismo. Pueden tener lesiones de la sustancia blanca en la RM cerebral (Marques et al, 1999) y presentar ataxia, sordera y disartria. Las mujeres suelen tener cuadros más leves y de inicio más tardío, aunque no siempre. La conducción motora es más lenta en los hombres (CMT intermedio) y suele estar en el rango axonal de las velocidades en mujeres (Birouk et al, 1998).

El resto de formas recesivas ligadas al cromosoma X son muy raras y cursan con cuadros similares. La enfermedad CMT recesiva ligada al cromosoma X en Xp22.2 se conoce como CMTX2 (Ionasescu et al, 1991). CMTX3 es causado por un reordenamiento genómico entre los cromosomas 8q24.3 y Xq27.1 y ha sido descrito más extensamente en pacientes pediátricos australianos (Kanhangad et al, 2018). El síndrome de Cowchock, que se asigna al cromosoma Xq26, también se conoce como CMTX4 (Cowchock et al, 1985). CMTX5 está causado por una mutación en el gen *PRPS1* en el cromosoma Xq22 y se caracteriza por una severa neuropatía y atrofia óptica con sordera (Rosenberg y Chutorian, 1967). CMTX6 está causado por una mutación en el gen *PDK3* en Xp22 (Kennerson et al, 2013).

MANEJO TERAPÉUTICO

Desde que se publicaran los resultados del último ensayo clínico de pacientes pediátricos con NH (Burns et al, 2009), son múltiples los enfoques terapéuticos dirigidos a modificar la historia natural de la enfermedad en la última década. Entre éstos se incluyen el silenciamiento de genes y las terapias de reemplazo

génico, así como los tratamientos de moléculas pequeñas que se encuentran actualmente en pruebas preclínicas y varios han alcanzado la etapa de ensayo clínico. Algunos de los enfoques de tratamiento son específicos de la enfermedad dirigidos al mecanismo único de la enfermedad de cada forma de CMT, mientras que otras terapias se dirigen a las vías comunes compartidas por varios o todos los tipos de CMT (Stavrou et al, 2021).

No obstante, hasta que las terapias modificadoras de enfermedad sean una realidad clínica, el pilar fundamental del manejo de las NH sigue enfocado a mejorar la calidad de vida, la capacidad funcional y el dolor así como prevenir complicaciones (Fridman y Reilly, 2015). Se debe realizar una vigilancia periódica multidisciplinar del estado de la enfermedad y anticiparse a la posible progresión para implementar intervenciones que estén dirigidas a la preservación de la calidad de vida de los pacientes pediátricos. Recientemente ha salido a la luz la guía de práctica clínica para el manejo de CMT pediátrico (Yiu et al, 2022). De forma práctica, el manejo puede ser subdividido en los siguientes aspectos: terapia física y ocupacional, tratamiento ortopédico y manejo del dolor.

a) Terapia física y ocupacional

Se deben implementar regímenes de ejercicio y de estiramiento para mantener la función, reducir la atrofia por desuso y evitar las contracturas tendinosas, así como no producir fatiga por sobreesfuerzo. A la eficacia del entrenamiento de la musculatura proximal (Chetlin et al., 2004) se ha añadido recientemente el beneficio claro del entrenamiento de la musculatura distal. Así pues, se demostró que ejercicios de resistencia progresivos en la musculatura distal de niños con CMT consiguió mitigar la progresión de la debilidad en la dorsiflexión sin efectos deletéreos o debilidad por sobreesfuerzo en los entrenamientos de resistencia medida por RM (Burns et al., 2017). El efecto beneficioso del entrenamiento de resistencia se vio a los 24 meses, pero no a los 6 ni a los 12 meses.

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La actividad física regular debe ir enfocada al fortalecimiento, rango de movimiento y entrenamiento del equilibrio para mantener la movilidad de los pacientes (Burns et al., 2009). La natación y otras terapias de piscina pueden ser útiles para mantener la fuerza axial y prevenir la escoliosis. El papel de la terapia ocupacional es ofrecer herramientas para llevar a cabo las actividades de la vida cotidiana, particularmente las relacionadas con la manipulación que ayuden a los niños en las tareas escolares (Matyjasik-Liggett et al., 2013).

b) Tratamiento ortopédico

La displasia de cadera y la escoliosis ocurre hasta en un 8 y un 38% de los individuos con CMT, respectivamente, causando frecuentemente dolor de espalda y de cadera (Walker et al., 1994a y b). Para la displasia de cadera se suele precisar de intervenciones ortopédicas especializadas, pero raramente se necesitan para las escoliosis a no ser que la deformidad evolucione rápidamente más allá de los 45° (Rossor et al., 2015).

Para pacientes con debilidad de la musculatura del pie y del tobillo se pueden usar ortesis anti-equinas (DAFOs), las férulas o el calzado especializado en función de la gravedad de la debilidad o la distribución de cargas (Ramdharry et al., 2012). Un DAFO articulado cuenta con unas ventajas y desventajas distintas a uno sólido por lo que su elección dependerá de la presentación clínica (Phillips et al., 2012). En los últimos años se ha descrito que la impresión 3D se puede utilizar para replicar DAFOs tradicionales hechos a mano y para rediseñar DAFOs para producir un dispositivo más liviano con biomecánica mejorada al incorporar características de diseño novedosas (Wojciechowski et al., 2022).

Si el tratamiento conservador de las deformidades de pie con plantillas ortopédicas fallase, será necesario su abordaje quirúrgico el cual incluye: la transferencia de tendón, fasciotomía, osteotomías, artrodesis y/o alargamiento del tendón de Aquiles (Yagerman et al., 2012; Reilly et al., 2017). Sin embargo, la variabilidad en el abordaje quirúrgico es considerable y hasta la fecha no hay guías clínicas sobre el manejo quirúrgico óptimo de las deformidades de pie (Reilly et al., 2017).

c) Manejo del dolor

Para manejar adecuadamente el dolor es esencial identificar el origen del mismo como neuropático o músculoesquelético (Skre, 1974). En las NH el dolor generalmente está relacionado con problemas funcionales y estructurales en los miembros inferiores y/o calambres musculares (Crosbie et al., 2008). Así pues, el manejo del dolor es complejo y se debe complementar con la terapia física y un abordaje ortopédico. La formación de callos dolorosos en la planta del pie se relaciona normalmente con una distribución anormal de la presión (Crosbie et al., 2008). El uso de plantillas ortopédicas o calzado ortopédico pueden ayudar a redistribuir los puntos de presión anormal, aunque, en ocasiones, será necesario recurrir a la cirugía en última instancia.

Los calambres musculares son muy frecuentes en el grupo de los gastrocnemios y a menudo se relacionan con contracturas en el tobillo y con la marcha de puntillas. Es por ello que los estiramientos, el uso de dispositivos ortopédicos y las intervenciones quirúrgicas son a veces necesarias para el manejo del dolor. El manejo médico del dolor y de los calambres es subóptimo. La gabapentina, pregabalina, benzodiazepinas y AINEs pueden ser útiles durante un corto periodo de tiempo pero su uso crónico es problemático (Johnson et al., 2015).

B. MEDIDAS DE EVALUACIÓN EN LA ENFERMEDAD DE CHARCOT-MARIE-TOOTH PEDIÁTRICA

Las medidas de evaluación, o *outcome measures*, han de ser validadas clinimétricamente y además se tienen que ser bien toleradas, validas, fehacientes y sensibles (Reilly et al., 2012). Así mismo, estos marcadores de progresión deben permitir detectar cambios en la evolución de la enfermedad, sin verse influidos por factores ajenos a la misma, y poder así monitorizar la respuesta a la terapia.

INTRODUCCIÓN

El desarrollo de terapias racionales que persiguen alterar la historia natural del CMT es ya una realidad (Stavrou et al, 2021). El inicio de ensayos clínicos en pacientes pediátricos está en el horizonte próximo. Así pues, será necesario caracterizar en profundidad las medidas de evaluación en esta población para diseñar con éxito ensayos clínicos en la infancia.

ANTECEDENTES

En los adultos con CMT (mayores de 16 años), la *CMT Neuropathy Score* (CMTNS) fue la establecida como la medida de evaluación primaria en numerosos ensayos clínicos (Micallef et al., 2009; Pareyson et al., 2011). La CMTNS presenta una puntuación compuesta basada en los síntomas del paciente, el examen neurológico, las limitaciones en algunas actividades y parte del resultado del ENG. En un ensayo clínico reciente de vitamina C en adultos con CMT1A, la versión original de la escala de discapacidad para adultos (CMTNSv1) fue incapaz de detectar progresión de enfermedad en 2 años por lo que la CMTNS no resultó útil para monitorizar la respuesta a la vitamina C (Piscosquito et al., 2015). En este mismo trabajo se concluyó que los resultados dinamométricos de la presión manual y de la dorsiflexión del pie sí que fueron sensibles a la progresión de la enfermedad por lo que se consideró que sería interesante retener estos ítems en ensayos clínicos futuros.

La CMTNSv1 mostró una sensibilidad limitada para distinguir el nivel de gravedad en niño/as con CMT, en concreto en los que padecen CMT1A (Haberlová y Seeman, 2010). Esta primera versión de la escala fue modificada, manteniendo los mismos ítems, con el fin de mejorar la fiabilidad y la sensibilidad (CMTNSv2) (Murphy et al., 2011). No obstante, seguía sin existir una escala validada que midiese la discapacidad en niños y tuviese en cuenta la influencia del crecimiento.

MEDIDAS DE EVALUACIÓN PARA POBLACIÓN PEDIÁTRICA

En el 2012, la escala diseñada para medir la discapacidad en CMT en la población pediátrica, conocida como *CMT Pediatric Scale* (CMTPedS), fue validada para los pacientes de entre 3 y 20 años (Burns et al., 2012). Esta escala incluye medidas de destreza manual, fuerza, sensibilidad, marcha, equilibrio, fuerza y resistencia. Además, se demostró que la puntuación total en la CMTPedS se correlacionaba con la obtenida mediante la CMTNSv2, especialmente en los adolescentes y adultos jóvenes, apoyando así la transición entre estas dos escalas para medir discapacidad en los pacientes con CMT a lo largo de toda su vida (Burns et al., 2013).

La escala pediátrica CMTPedS ofrece la posibilidad de comparar objetivamente los pacientes de diferentes centros (y así desarrollar estudios de colaboración internacionales), conocer la historia natural de la enfermedad desde el inicio de la misma, comparar la gravedad en pacientes con mutaciones en un mismo gen y en pacientes con diferente genotipo. Cuando la presente tesis fue planteada, la gravedad de la enfermedad de CMT en la infancia sólo se había descrito en algunas formas raras graves (Sevilla et al., 2003; Sevilla et al., 2011) pero no había sido caracterizada en series pediátricas largas con el mismo genotipo ni tampoco entre diferentes genotipos. Un estudio multicéntrico que incluyó a 520 participantes de 3-20 años analizó la variabilidad de la gravedad en los subtipos más frecuentes de su cohorte: CMT1A, CMT2A, CMT1B, CMT4C y CMTX1 (Cornett K et al., 2016). Comprando los resultados en la CMTPedS comprobaron que los subtipos CMT1B, CMT2A y CMT4C mostraban un fenotipo más grave que los pacientes con CMT1A y CMTX1. Así mismo, la CMTPedS ha demostrado detectar progresión de forma significativa a los dos años en pacientes con CMT1A con una tasa de progresión de 1.8 ± 4.2 (Cornett K et al., 2017).

Aún resta por averiguar si la CMTPedS sería capaz de detectar progresión de enfermedad en un año en los pacientes con CMT1A o si detectaría diferencias entre otros subtipos de CMT que son más prevalentes en nuestra área como los

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CMT asociados a mutaciones monoalélicas en el gen *GDAP1* (Sivera et al., 2013).

2. JUSTIFICACIÓN

JUSTIFICACIÓN

Las NH, siendo enfermedades raras, son las enfermedades neuromusculares más prevalentes en niños (Ouvrier y Nicholson, 1995). La heterogeneidad genética y la complejidad biológica de las mismas plantean continuos retos a la hora del diagnóstico. Actualmente no existe tratamiento curativo para las neuropatías hereditarias por lo que el abordaje terapéutico se limita al consejo genético, tratamiento sintomático y rehabilitación. Antes de la llegada de las técnicas de secuenciación masiva la posibilidad de éxito mediante el estudio de genes candidatos era escasa y el coste económico, muy alto (Baets et al., 2011). Estas técnicas han revolucionado el diagnóstico genético, pero también nos ponen retos importantes debido a los numerosos cambios detectados.

La justificación de la presente tesis se basa en que los estudios de series clínicas largas permiten determinar la epidemiología genética, establecer los límites del fenotipo (delinear fenotipos) y explorar el solapamiento entre neuropatías hereditarias. Actualmente los avances en genética molecular con las nuevas técnicas de secuenciación masiva permiten una mayor exactitud diagnóstica pero el fenotipado en profundidad sigue siendo fundamental para interpretar la gran cantidad de resultados generados (Laurá et al., 2019).

Otra razón que apoya la necesidad del presente trabajo es el inminente inicio de ensayos clínicos en la población pediátrica lo cual implica que debemos profundizar en las medidas de evaluación y tener a los candidatos bien caracterizados clínica y genéticamente de cara a las nuevas terapias racionales. Se ha demostrado la capacidad de la escala CMTPedS de detectar significativamente la progresión en dos años en el CMT1A (Cornett et al., 2017). Es previsible que nuestra serie pediátrica presente características diferenciadoras con otras cohortes descritas tal y como demostró el estudio de la cohorte de adultos de nuestra región (Sivera et al., 2013). Así pues, el estudio prospectivo de la presente serie nos permitirá ahondar en la utilidad de la CMTPedS para detectar la variabilidad fenotípica y la progresión en distintos subtipos de NH.

3. OBJETIVOS

OBJETIVOS

OBJETIVOS

El objetivo principal de la presente tesis es la caracterización genética y fenotípica de una serie de pacientes menores de 20 años con NH incluyendo la búsqueda de nuevos genes responsables.

Los objetivos secundarios son los siguientes:

1. Determinar la variabilidad de la gravedad de los diferentes tipos de NH.
2. Estimar la sensibilidad de la escala CMTPedS como medida de progresión en los diferentes subtipos genéticos de NH de cara a los ensayos clínicos.

OBJETIVOS

4. METODOLOGÍA

METODOLOGÍA

A. TIPO DE ESTUDIO

Se trata de un estudio descriptivo longitudinal de 3 años de duración de una serie hospitalaria de pacientes menores de 20 años con NH. Estos pacientes fueron reclutados y valorados de forma prospectiva entre septiembre 2017 y septiembre de 2020 en consultas externas de la Unidad de neuromuscular del Hospital Universitario y Politécnico La Fe (HLF).

B. SELECCIÓN DE PACIENTES

ÁMBITO DE ESTUDIO

La cohorte principal procede de la Unidad de Enfermedades Neuromusculares y Neuropediatría del HLF. Se trata de una Unidad de Referencia, CSUR, del Sistema Nacional de Salud para pacientes adultos y pediátricos. Para el estudio centrado en la utilidad de la escala CMTPedS en las formas motoras puras, contamos con la aportación de pacientes por parte del grupo de investigación en neuropatías hereditarias en edades pediátricas del *Children's Hospital at Westmead* (Universidad de Sídney, NSW, Australia). Los criterios de inclusión y exclusión que debían cumplir los pacientes procedentes de estos dos centros eran los mismos.

CRITERIOS DE INCLUSIÓN

Se incluyeron solo los pacientes que tenían menos de 20 años en el momento del inicio del estudio y contaban con un diagnóstico definitivo de neuropatía periférica de origen genético, aunque no se conociese el defecto genético causal. Los pacientes reclutados podían ser casos índices o secundarios (algún

progenitor o hermano/a afectos). En el supuesto de que no hubiese historia familiar de NH (caso esporádico) se consideró que la causa era genética si la historia natural (de inicio temprano y progresión lenta), la clínica y los resultados neurofisiológicos eran compatibles con NH y se habían excluido otras causas de neuropatía por medio de pruebas de laboratorio o por el contexto clínico.

CRITERIOS DE EXCLUSIÓN

Se excluyeron aquellos pacientes afectos de neuropatía hereditaria con predisposición a las parálisis por compresión, así como aquellos con AME debida a mutaciones bialélicas en el gen *SMN1*. Estos supuestos clínicos no se incluyeron en el presente trabajo por presentar una historia natural y una base genética muy particular y distinta al resto de afectaciones del nervio periférico.

No se incluyeron tampoco aquellos casos clínicos en los que se sospechaba o se confirmó causa adquirida. Por un lado, se excluyeron los pacientes con diagnóstico definitivo de polineuropatía desmielinizante inflamatoria tanto aguda como crónica y aquellos cuya neuropatía respondía a tratamiento inmunomodulador o inmunosupresor. Por otro, también se excluyeron los pacientes con neuropatía periférica (generalmente axonal) diagnosticada en contexto de enfermedad del paciente crítico, toxicidad por fármacos (u otros) o de enfermedades sistémicas no genéticas como la diabetes.

C. CARACTERIZACIÓN CLÍNICA O FENOTIPADO

Los pacientes se dividieron en los dos grandes fenotipos expuestos en la introducción de la presente tesis:

- **NH primarias** cuando la manifestación principal fue la neuropatía periférica, aunque tuvieran signos leves de afectación de vía piramidal o cerebelo. A su vez, los subdividimos según los hallazgos de ENG en:
 - **Sensitivo-motoras** (NHSM o CMT):
 - Desmielinizantes (CMT1) cuando la VC motora en un nervio de la extremidad superior es < 38 m/s siempre que la amplitud del potencial de acción motor compuesto de ese mismo nervio no esté disminuido más del 80% del límite inferior de la normalidad. En esos casos, para catalogar el fenotipo de desmielinizante se determinaron las velocidades de conducción a los músculos proximales (palmaris longus para el nervio mediano, flexor cubital del carpo para el nervio cubital, etc.), y, ocasionalmente, las latencias de otros nervios más cortos como el axilar, o se tuvo en cuenta los hallazgos patológicos.
 - Axonales (CMT2) si la VC motora en un nervio de la extremidad superior es > 38 m/s.
 - **Motoras puras** (NHM) que subdividimos en NHMd y AME no5q en función de si el patrón era o no dependiente de longitud, respectivamente.

- **NH complejas** si el cuadro clínico se podía englobar en una enfermedad metabólica y la neuropatía periférica no era la que dominaba el mismo.

Para perfilar el fenotipo nos apoyamos tanto en los hallazgos de la exploración neurológica como en los neurofisiológicos y de imagen muscular. A continuación, se detallan los protocolos seguidos en estos tres pilares del fenotipado.

EXPLORACIÓN NEUROLÓGICA

En el HLF la evaluación clínica de los pacientes con neuropatías hereditarias está protocolizada, de manera que incluye un cuestionario detallado sobre los síntomas relacionados con NH y una exploración física detallada. Para detectar formas clínicas leves, se exploraron de forma sistemática a familiares de interés como son los progenitores, hermanos/as y parientes sintomáticos. Estas evaluaciones a los pacientes se realizaron anualmente, como mínimo, e incluyeron las valoraciones de los siguientes aspectos:

- **Función motora:** compuesta por una gradación de la fuerza de los grupos musculares principales utilizando la escala estandarizada del *Medical Research Council* (MRC) y la apreciación de atrofiás musculares en extremidades. La MRC es una escala ampliamente utilizada y validada que puntúa de 0-5 la fuerza en cada grupo muscular (Florence et al. 1992).
- **Función sensitiva:** incluye la exploración de la sensibilidad táctil fina, algésica, vibratoria y posicional de las extremidades. Para explorar la sensibilidad vibratoria se empleó un diapasón Rydel-Seiffer de 128 Hz graduado de 0 a 8, indicando 0 la ausencia de percepción mientras que 8 es la percepción máxima de la vibración.

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- La presencia o ausencia de los reflejos osteotendinosos (ROT) y la respuesta cutáneo plantar (RCP).
- Deformidades asociadas: Tanto distales (pie cavo, dedos en martillo, retracción aquílea, manos en garra, etc.) como proximales (escoliosis, etc.).
- Otros signos pertenecientes a la afectación de otras estructuras neurológicas: vía piramidal, cerebelo, sistemas vestibular, auditivo y visual.

PROTOCOLO NEUROFISIOLÓGICO

Los estudios neurofisiológicos se realizaron a todos los individuos presentes en esta tesis en al menos una ocasión y a algunos de los progenitores u otros familiares en función de los hallazgos a la exploración neurológica para definir mejor la forma de presentación y el patrón de herencia. Todas las pruebas neurofisiológicas fueron llevadas a cabo en el Servicio de Neurofisiología Clínica del HLF y en el Departamento de Neurología de *The Children's Hospital at Westmead* por profesionales con amplia experiencia en patología neuromuscular siguiendo el mismo protocolo. Los estudios de conducciones nerviosa (ENG) sensitivas y motoras con electrodos de superficie y la electromiografía con aguja concéntrica (EMG) se ejecutaron con el dispositivo Medelec Synergy (Viasys Healthcare, Conshohocken, PA, USA). Siempre se siguió el mismo protocolo resumido a continuación y se mantuvo una temperatura cutánea en manos y pies entre 30 y 32°C (Sevilla et al., 2003).

En el ENG los registros electrofisiológicos fueron obtenidos de los siguientes nervios motores: peroneal, tibial posterior, cubital, mediano y axilar; y sensitivos: sural (antidrómico), mediano y radial (ortodrómico ambos). Los parámetros

evaluados fueron: latencia motora distal (LMD), VCN motora y sensitiva, amplitudes de los potenciales de acción nerviosa motores compuestos y sensitivos (PAMCs y PANSs, respectivamente) las cuales se midieron de la línea de base al pico negativo.

La VCN motora del nervio peroneal se obtuvo tras estimulación eléctrica a nivel de la fosa poplítea y tobillo y recogiendo el PAMC en el músculo *tibialis anterior* (TA) y *extensor digitorum brevis* (EDB), respectivamente. La VCN motora del nervio tibial posterior se obtuvo registrando el PAMC en el *abductor hallucis* (AH). La VCN motora de los nervios mediano y cubital se midió tras estimulación eléctrica supramáxima en el codo y en la muñeca, con la recogida del PAMC en los músculos *abductor pollicis brevis* (APB) y *abductor digiti minimi* (ADM), respectivamente. Si la amplitud del PAMC a músculos distales fue $< 0,5$ mV, se recogió la medida a músculos más proximales (palmar largo, flexor cubital del carpo, etc.). Para evaluar las LMDs se emplearon distancias estandarizadas. Asimismo, se evaluaron la presencia de bloqueos y/o dispersión del PAMC. Las ondas F se valoraron en los nervios motores medianos, cubital y tibial posterior.

Para la interpretación de los resultados del ENG se emplearon estudios recientes en población pediátrica y se consideró anormal los valores de amplitudes y VCN $< 5^{\circ}$ percentil y los valores de LDM $>95^{\circ}$ percentil para la edad del sujeto (Ryan et al., 2019).

El estudio EMG incluyó un registro de la actividad muscular en reposo, con contracción sostenida y con esfuerzo máximo en al menos dos grupos musculares de cada extremidad.

RESONANCIA MAGNÉTICA MUSCULAR

La resonancia magnética muscular de cuerpo entero no se realizó de manera rutinaria, sino que se seleccionaron aquellos pacientes con NH asociada a genes

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muy poco frecuentes y en los que podía aportar información relevante para delimitar el fenotipo, así que en los pacientes con CMT1A no se realizó, por ejemplo. El protocolo fue diseñado expresamente para pacientes con neuropatías e incluye cortes axiales y longitudinales de las extremidades inferiores (incluyendo pies), extremidades superiores y cinturas pelviana y escapular. Se empleó un sistema de 3-Teslas (Siemens Vision, Erlangen, Alemania) y se realizaron secuencias potenciadas en T1, T2 y STIR (*Short T1 Inversion Recovery*). Se realizó una valoración cualitativa de los grupos musculares afectados para la búsqueda de patrones de afectación en función de ciertos genotipos, pero no se llevó a cabo un estudio semicuantitativo de forma sistemática.

VALORACIÓN DE LA DISCAPACIDAD

Para el estudio de la variabilidad de la gravedad fenotípica y el grado de progresividad hasta dos años vista se empleó la escala pediátrica de discapacidad CMTPedS en los mayores de 3 años. La CMTPedS fue administrada por la misma evaluadora (HAE) en todos candidatos procedentes del HLF en, al menos, tres ocasiones en cada paciente: basal, al año y a los dos años. En el subestudio enfocado en las formas motoras puras, los niños y adolescentes pertenecientes al *Children's Hospital at Westmead* fueron evaluados por examinadores capacitados de dicho centro, lugar en el que la CMTPedS fue diseñada.

La CMTPedS incluye 7 áreas de valoración y 11 ítems en total (Burns et al., 2012):

- Habilidad manual: prueba de destreza funcional y test de los 9 agujeros (*9-Hole-Peg Test*).
- Fuerza de la prensión manual y la flexión plantar y dorsiflexión del tobillo cuantificadas con dinamómetro manual *Hand-Held Dynamometry CITEC®*.

- Sensibilidad de las extremidades inferiores: al pinchazo y la vibración medida con diapason graduado.
- Marcha: dificultad para caminar de talones y de puntillas y presencia de pie caído.
- Equilibrio medido con la prueba de Bruininks-Oseretsky de competencia motora, 2ª ed), (Bruininks y Bruininks, 2005).
- Impulso: salto de longitud.
- Resistencia: test de los 6 minutos.

Figura 4. Plantilla que recoge las puntuaciones brutas para cada uno de los ítems que componen la escala pediátrica de CMT (CMTPedS).

Hand Dexterity											
1. Functional Dexterity Test (sec)					2. Nine-hole peg test (sec)						
Strength		Trial 1		Trial 2		Trial 3		Average			
3. Hand grip (N)								x2:			
4. Foot plantarflexion (N)											
5. Foot dorsiflexion (N)											
Sensation	0	1	2	3	4	Score					
6. Pinprick	Normal	Decreased below or at ankle bones	Decreased at or below midline of calf	Decreased above calf midline up to and including knee	Decreased above knee (above top of patella)						
7. Vibration	Normal	Reduced at first metatarsal bone	Reduced at ankle	Reduced at knee (tibial tuberosity)	Absent at knee and ankle						
Balance		Assistive device required (e.g. AFO) Y/N. Describe device and footwear:									
8. Bruininks Oseretsky Test		Raw Score		Conduct second trial only if examinee does not earn the maximum score on the first trial						Point score	
		Trial 1	Trial 2	Raw	0.0-0.9	1.0-2.9	3.0-5.9	6.0-9.9	10		
Standing with feet apart on a line-eyes open				Point	0	1	2	3	4		
Walking forward on a line				Raw	0	1-2	3-4	5	6		
				Point	0	1	2	3	4		
Standing on one leg on a line-eyes open				Raw	0.0-0.9	1.0-2.9	3.0-5.9	6.0-9.9	10		
				Point	0	1	2	3	4		
Standing with feet apart on a line-eyes closed				Raw	0.0-0.9	1.0-2.9	3.0-5.9	6.0-9.9	10		
				Point	0	1	2	3	4		
Walking forward heel-to-toe on a line				Raw	0	1-2	3-4	5	6		
				Point	0	1	2	3	4		
Standing on one leg on a line-eyes closed				Raw	0.0-0.9	1.0-2.9	3.0-5.9	6.0-9.9	10		
				Point	0	1	2	3	4		
Standing on one leg on a beam-eyes open				Raw	0.0-0.9	1.0-2.9	3.0-5.9	6.0-9.9	10		
				Point	0	1	2	3	4		
Standing heel-to-toe on a balance beam				Raw	0.0-0.9	1.0-2.9	3.0-5.9	6.0-9.9	10		
				Point	0	1	2	3	4		
Standing on one leg on a beam-eyes closed				Raw	0.0-0.9	1.0-2.9	3.0-4.9	5.0-7.9	8.0-9.9	10	
				Point	0	1	2	3	4	5	
<small>Balance Subscale from the Bruininks Oseretsky Test of Motor Proficiency, Second Edition (BOT-2). Copyright© 2005 NCS Pearson, Inc. Adapted and reproduced with permission. All rights reserved.</small>											
Total											
Motor Function		Assistive device required (e.g. AFO) Y/N. Describe device and footwear:									
9. Gait		Foot drop: No <input type="checkbox"/> Some <input type="checkbox"/> Yes <input type="checkbox"/>			Difficulty heel walking: No <input type="checkbox"/> Some <input type="checkbox"/> Yes <input type="checkbox"/>			Difficulty toe walking: No <input type="checkbox"/> Some <input type="checkbox"/> Yes <input type="checkbox"/>			
10. Long jump (cm)					11. Six-minute walk test (m)						
Item Scores (0-4)											
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	Total Score (0-44)

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Más detalles sobre cómo evaluar cada uno de los ítems y el material necesario para ello se encuentran disponibles en <http://cmtpedS.org>.

Se evaluaron todos los ítems y las puntuaciones brutas se ajustaron por edad y sexo en función de los valores normativos de referencia procedentes del *1000 Norms Project* (McKay et al., 2017a; McKay et al., 2017b) para obtener puntuaciones z. Las puntuaciones Z se clasifican en categorías de acuerdo con una escala Likert que varía desde 0 (no afectado) a 4 (gravemente afectado). Una puntuación de categoría de 0 indica una puntuación z dentro de 1 desviación estándar (DE) de la media del valor de referencia normativo. Una puntuación de categoría de 1, 2 o 3 representa una puntuación z de 1 a 2, de 2 a 3 o de 3 a 4 DE por debajo de lo normal, respectivamente. Una puntuación de 4 representa más de 4 DE por debajo de lo normal. Los participantes que no pudieron realizar un ítem debido a la gravedad de la enfermedad recibieron una puntuación de 4. Los participantes que no pudieron realizar un ítem por otras razones (por ejemplo, lesión aguda, cirugía reciente, problemas de comportamiento) no fueron calificados y no se calculó una puntuación total. Estas puntuaciones categorizadas (de 0 a 4) se suman para dar lugar a una puntuación total CMTPedS que varía desde 0 a 44 (la puntuación más alta indica el fenotipo más grave). Si un paciente recibe una puntuación dentro del rango 0-14 se considera que se encuentra levemente afectado, mientras que se considerará que su fenotipo es moderado si puntúa entre 15-29 o grave si puntúa 30 o más. El programa necesario para la conversión de la puntuación de cada ítem y la obtención de una puntuación total es de acceso gratuito en <http://cmtpedS.org>.

D. CARACTERIZACIÓN GENÉTICA

ESTRATEGIA DIAGNÓSTICA

Los análisis genéticos se llevaron a cabo con la colaboración del grupo de Genética y Genómica de Enfermedades Neuromusculares perteneciente al programa de genética y enfermedades raras del Centro de Investigación Príncipe Felipe. Dicho grupo está liderado por la Dra. Carmen Espinos e integrado por el Dr. Vincenzo Lupo y otros colaboradores que han realizado el trabajo de secuenciación tipo Sanger y del panel de genes y han intervenido estrechamente en la interpretación de los resultados genéticos presentes en esta tesis.

La extracción de las muestras de ADN a partir de sangre periférica, su cuantificación y su control de calidad previo a su procesamiento para el análisis genético, se realizó en el Biobanco La Fe y en la Unidad de Genética y Genómica de Enfermedades Neuromusculares y Neurodegenerativas del Centro de Investigación Príncipe Felipe.

La estrategia que diseñamos para el estudio de las NH en la edad pediátrica fue la siguiente:

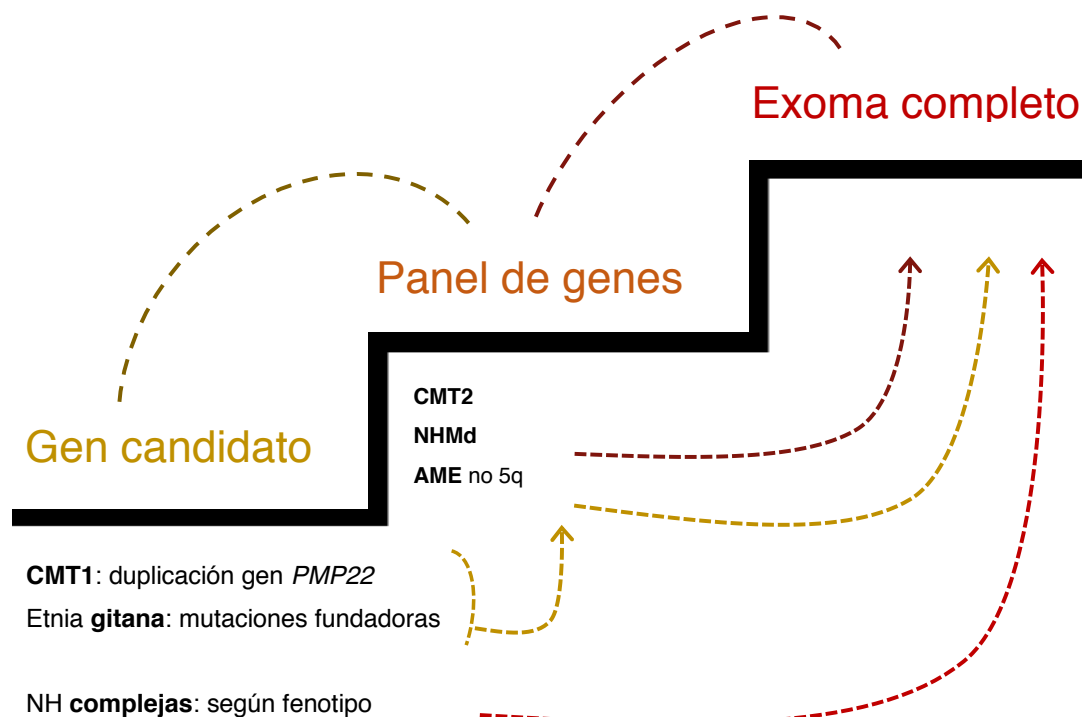
- a. Se llevó a cabo el estudio de la **duplicación CMT1A** a todos los pacientes con formas desmielinizantes (CMT1) por medio de la técnica MLPA (*Multiplex ligation-dependent probe amplification*).
- b. Se realizó estudio directo de otro/s gen/es candidato/s (mediante secuenciación tipo Sanger) en los siguientes supuestos:
 - Individuos de población romaní con NHSM que se presentaron como casos esporádicos o con sospecha de herencia recesiva (consanguineidad). En este supuesto se analizaron las mutaciones

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fundadoras de los genes *SH3TC2* (p.Arg1109* y p.Cys737_Pro737delins*), *HK1* (g.9712G>C) y *NDGR1* (p.Arg148*).

- Pacientes con NH secundarias o complejas cuando el fenotipo lo sugiera se secuenció el gen que cuadró con ese cuadro clínico.
- c. **Panel de genes:** se estudiaron así, por un lado, directamente las formas motoras puras (NHMd/AME no 5q) y las axonales (CMT2) como, por otro, los pacientes de etnia gitana negativos para las mutaciones fundadoras y las formas desmielinizantes (CMT1) que fueran negativas para la duplicación.
- d. **Estudio de exoma completo** para identificación de genes muy infrecuentes o no asociados a NH en la literatura. A este estudio se sometieron los pacientes no diagnosticados genéticamente en los pasos previos.

Figura 5. Representación de la estrategia para el diagnóstico molecular en neuropatía periféricas en la infancia.



TIPOS DE ESTUDIO

a) MLPA

Se ha realizado el estudio molecular de la duplicación del locus CMT1/NHPP en el cromosoma 17, que incluye el gen PMP-22 en ADN del paciente mediante la técnica de MLPA (Multiplex Ligation-dependent Probe Amplification) para detectar el nº de copias de la región CMT1/NHPP. Se empleó la salsa comercial P033-B4 CMT1 (MRC-Holland, Amsterdam, Países Bajos) realizándose el análisis mediante el programa informático Coffalyser. Esta técnica fue llevada a cabo por el personal de la Unidad de Genética de nuestro centro, Hospital Universitari y Politècnic La Fe.

b) Secuenciación tipo Sanger

Se empleó esta técnica para la validación de mutaciones puntuales en genes concretos y para el análisis de segregación en familiares sanos y enfermos. El análisis de los exones codificantes y regiones intrónicas flanqueantes de dichos genes se ha realizado mediante reacción en cadena de la polimerasa (PCR) con cebadores específicos y posterior secuenciación automatizada mediante el método de *Sanger*. El diseño de cebadores y experimentos de PCR han sido realizado en colaboración con la Unidad de Genética y Genómica de Enfermedades Neuromusculares y Neurodegenerativas del Centro de Investigación Príncipe Felipe. Las reacciones de PCR se llevaron a cabo en termocicladores Veriti® Thermal Cycler (Applied Biosystems). Los productos de PCR se visualizaron empleando GelRed (Biotium), en geles de agarosa al 1%. La secuenciación Sanger se realizó en el Servicio de Genómica y Genética Traslacional (SGGT) del CIPF, en un secuenciador de 96 capilares ABI3730xl (Applied Biosystems). Las secuencias de interés se analizaron mediante alineamiento de secuencias con el programa tblastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=tblastn&PAGE_TYPE=BlasSearch&LINK_LOC=blasthome) y mediante la visualización del

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electroferograma en el programa Sequence Analysis software version 5.4 (Applied Biosystems).

c) Panel de genes

Se analizó mediante secuenciación masiva las regiones codificantes e intrónicas flanqueantes (± 25 nucleótidos) de 120 genes asociados al CMT, NHMd y AME no 5q (véase más abajo). El sistema de captura empleado consiste en un enriquecimiento por hibridación en solución con el kit SureSelect QXT (Agilent Technologies) de diseño propio, que ha sido realizado con la herramienta SureDesign de Agilent Technologies (<https://earray.chem.agilent.com/suredesign/>). La secuenciación ha sido realizado en un equipo MiSeq de Illumina. La fase experimental de dicha técnica se realizó por el mencionado equipo del Centro de Investigación Príncipe Felipe.

Para la selección de variantes candidatas a ser responsables de la enfermedad se han tenido en cuenta los siguientes parámetros: calidad de alineamiento y cobertura ($>10x$); la frecuencia alélica en población control teniendo principalmente en cuenta las bases de datos del ExAC y gnomAD (excluyendo variantes con frecuencia alélica >0.01); el posible impacto de la variante sobre el mRNA y/o la proteína; el modo de herencia del gen que porta el cambio genético y su correlación con el fenotipo del probando. Tanto la validación de las variantes genéticas candidatas como el estudio de segregación en familiares sanos y enfermos, ha sido realizada mediante la técnica de *Sanger*, en un secuenciador de 96 capilares ABI3730xl (Applied Biosystems).

La interpretación de variantes candidatas y su clasificación final se realizó entre facultativos especialistas y genetista y siguiendo las guías del *American College of Medical Genetics* (Richards et al, 2015).

A continuación, se detallan los 120 genes relacionados con fenotipos CMT, NHMd y AME no 5q que se incluyeron en mencionado panel:

AARS, AIFM1, AIMP1, ALS2, ANG, ARHGEF10, ARHGEF28, ASAH1, ATP7A, BICD2, BSCL2, C12ORF65, CCNF, CHCHD10, CHMP2B, COX6A1, DAO, DCAF8, DCTN1, DCTN2, DGAT2, DHTKD1, DNAJB2, DNAJC6, DNMT2, DNMT1, DRP2, DYNC1H1, EGR2, ERBB4, FBLN5, FBXO38, FGD4, FIG4, FUS, GAN, GARS, GDAP1, GJB1, GJB3, GLE1, GNB4, HADHB, HARS, HINT1, HNRNPA1, HNRNPA2B1, HSPB1, HSPB3, HSPB8, IFRD1, IGHMBP2, INF2, KARS, KIF1A, KIF1B, KIF5A, KLHL9, LAS1L, LITAF, LMNA, LRSAM1, MARS, MATR3, MCM3AP, MED25, MFN2, MME, MORC2, MPZ, MTMR2, MYH14, NAGLU, NDRG1, NEFH, NEFL, NEK1, OPTN, PARK7, PDK3, PFN1, PLEKHG5, PMP2, PMP22, PNKP, PRPH, PRPS1, PRX, RAB7A, REEP1, SBF1, SBF2, SETX, SH3TC2, SIGMAR1, SLC12A6, SLC25A46, SLC5A7, SOD1, SPG11, SPTLC1, SPTLC2, SPTLC3, SQSTM1, SS18L1, SURF1, TAF15, TARDBP, TBK1, TFG, TRIM2, TRPV4, TUBA4A, TUBB3, UBA1, UBQLN2, VAPB, VCP, VRK1, YARS

d) Secuenciación de exoma completo

Los estudios de exoma completo (WES, *whole exome sequencing*) se realizaron con el apoyo del Centro Nacional de Análisis Genómico (CNAG-CRG, Barcelona). Para el filtrado y priorización de variantes se empleó una plataforma procedente de iniciativa europea conocida como *RD-Connect Genome-Phenome Analysis Platform*.

Para la fragmentación Del ADN y el enriquecimiento del exoma se empleó el kit SureSelect Human All Exon V5 (Agilent Technologies, Santa Clara, CA, EE. UU.) y se siguieron las instrucciones el fabricante. Las librerías capturadas se secuenciaron en la HiSeq4000 (Illumina) el final de ambos extremos de los fragmentos de ADN (*paired-end mode*) con una profundidad de lectura de 2x10¹ pares de bases (pb) para generar una profundidad de cobertura de una mediana mínima de 125x. El análisis de la imagen, el llamamiento de las bases y la

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puntuación de la calidad de lo secuenciado fueron procesados usando el programa Real Time Analysis (v 2.7.7) y seguidamente se generaron los archivos las secuencias con el formato FASTQ. El alineamiento de las secuencias y el llamamiento de las variantes se hizo frente a la versión del genoma humano de referencia (GRCh37) conocido como hs37d5 y se siguió de un protocolo bioinformático ya descrito previamente en el cual también se especificó la anotación y filtrado de las variantes (Laurie et al., 2016). Priorizamos las variantes encontradas en genes ya asociados a neuropatía. la clasificación de las variantes siguió el criterio del colegio americano de genética médica y genómica (ACMG, del inglés *American College of Medical Genetics and Genomics*). Las variantes no descritas o nuevas se conocieron patogénicas si: estaban presentes en un gen ya conocido como causante neuropatía, se predecía que fuese deletrea (nucleótido altamente conservado, la función o estructura proteica estaba alterada *in silico*), y segregaba con la enfermedad. todas las variantes nuevas no fueron encontradas en bases de datos de controles y mutaciones (ExAC, GnomAD, NCBI, ClinVAR, y HGMD). Las variantes patogénicas putativas fueron confirmadas por secuenciación tipo Sanger y se sometieron a estudios de segregación. Para los análisis *in silico* de las variantes se emplearon PROVEAN (<http://sift.jcvi.org/>), SIFT (<http://sift.jcvi.org/>), Polyphen2 (<http://genetics.bwh.harvard.edu/pph2/>), y MutationTaster (<http://www.mutationtaster.org/>).

LIMITACIONES DE ESTE ENFOQUE GENÉTICO

No conseguiremos alcanzar el diagnóstico genético en los siguientes supuestos:

- Cuando la/s mutación/es responsables no se localicen en los exones o regiones codificantes. En el panel de genes realizado sí que se secuenciaron las regiones intrónicas flanqueantes. No obstante, en el WES sólo se secuenciaron las regiones exónicas.

- Cuando la enfermedad del paciente se deba a mutaciones en el genoma mitocondrial.
- Cuando las alteraciones genéticas sean grandes deleciones o inserciones, expansiones nucleotídicas o fenómenos de variación del número de copias (duplicaciones o deleciones) distintas a la de la región cromosómica 17p11.2-12 (que es la asociada al CMT1A y sí fue estudiada empleando *MLPA*). Éstas son limitaciones inherentes a las técnicas de secuenciación masiva.

E. ANÁLISIS ESTADÍSTICO

Los datos fueron analizados utilizando el programa SPSS versión 22.0 (IBM Corp. Armonk, NY). A continuación, se describen cómo se mostraron los aspectos descriptivos del análisis de los datos. Las variables categóricas se muestran en valor absoluto y porcentaje. Las variables cuantitativas se describen mediante media y desviación estándar (DE) o mediante mediana y rango intercuartílico según presentaban o no, respectivamente, una distribución normal. Se empleó la prueba de Kolmogorov-Smirnov para estudiar la distribución de las variables cuantitativas.

La significación estadística del cambio en la puntuación total de CMTPedS y de cada uno de los ítems de la escala durante el período de un año y de dos años se calculó utilizando ANCOVA de medidas repetidas con el tiempo de seguimiento como co-variable y el p valor fue corregido con el método de Bonferroni. En todos los casos acepto un nivel de significación del 5% (p valor < 0.05).

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Para estudiar la utilidad de la escala CMTPedS en pacientes de 3 a 20 años con NHMd, se analizaron los siguientes aspectos sobre los resultados de las evaluaciones basales de la CMTPedS: correlaciones entre ítems tanto para puntuaciones como para puntuaciones z (determinados por coeficiente de correlación de Pearson), correlación de la puntuación z de los ítems con puntuación total de la CMTpedS (usando Pearson), y consistencia (calculada con α de Cronbach).

F. COMITÉ DE BIOÉTICA Y CONFIDENCIALIDAD

El estudio se realizó siguiendo las directrices de la declaración de Helsinki y contó con la aprobación del Comité Ético de Investigación Clínica del Hospital Universitario y Politécnico La Fe (número de registro 2017/0351). Todos los participantes o sus progenitores/tutores dieron su consentimiento informado por escrito.

El tratamiento, la comunicación y a cesión de los datos de carácter personal de todos los sujetos participantes, se ajustó a lo dispuesto en la Ley Orgánica 15/1999, del 13 de diciembre, de protección de datos de carácter personal. La responsable del registro de los datos en la Agencia Española de Protección de Datos fue la Conselleria de Sanitat. Las muestras de sangre utilizadas en el estudio fueron procesadas y conservadas en el Biobanco La Fe en cumplimiento con la ley orgánica de protección de datos.

5. RESULTADOS

RESULTADOS

En este apartado se resumen los resultados de los trabajos directamente relacionados con el proyecto de caracterización fenotípica y molecular de las neuropatías hereditarias en la infancia y la adolescencia y en los que la autora de la presente tesis doctoral actúa como autora principal.

En el estudio participaron un total de 110 pacientes con NH que en el momento del inicio mismo tenían menos de 20 años. La mayoría pertenecen a la cohorte de pacientes remitidos al Hospital Universitario y Politécnico La Fe o HLF mientras que 8 de ellos fueron aportados por *The Children's Hospital at Westmead* (NSW, Australia) o CHW. Colaboramos con el grupo de investigación liderado por el Dr. Burns y el Dr. Menezes de dicho complejo hospitalario perteneciente a la Universidad de Sídney para la caracterización más en profundidad de la subcohorte de 22 pacientes con NHMd (Argente-Escrig et al., 2021b). Los restantes 88 debutaron con una forma sensitivomotora (CMT), de los cuales tres presentaron un fenotipo tan único y compartían el mismo genotipo *TRMT5* que fueron descritos en un extenso trabajo aparte (Argente-Escrig et al., 2022).

Todos los pacientes fueron caracterizados clínica y genéticamente. A continuación, se detalla qué método genético fue empleado para conseguir el diagnóstico molecular de la cohorte del HLF entera (n=102) y clasificados en función del fenotipo.

RESULTADOS

Tabla 1. Método genético empleado para alcanzar el diagnóstico molecular de la cohorte del Hospital Universitari i Politècnic La Fe en función del fenotipo.

Metodología genética	Número de pacientes (% de cada subgrupo)			
	Cohorte entera (n=102)	CMT desmielinizante (n=66)	CMT axonal (n=22)	NMHD (n=14)
MLPA para <i>duplicación PMP22</i>	37 (36.3%)	37 (56.1%)	0 (0%)	0 (0%)
Panel de genes (NGS)	16 (15.7%)	9 (13.6%)	5 (22.7%)	2 (14.3%)
Estudio segregación (Sanger)	16 (15.7%)	4 (6.1%)	9 (40.9%)	3 (21.4%)
Estudio gen candidato (Sanger)	10 (9.8%)	5 (7.6%)	5 (22.7%)	0 (0%)
NGS WES	23 (22.5%)	11 (16.7%)	3 (13.6%)	9 (64.3%)

RESULTADOS

A. DISTRIBUCIÓN GENÉTICA Y CORRELACIÓN FENOTÍPICA DE LAS NH EN EDAD PEDIÁTRICA

La primera parte del primero de los artículos anexados (Argente-Escrig et al., 2021a) se centra en la descripción clínica, genética y la variabilidad de la gravedad de 99 pacientes con NH de nuestra cohorte exclusivamente (HLF). 85 mostraron NHSM (63 con formas desmielinizantes) y 14 padecían NHMd. Como anteriormente se mencionaba, en este primer trabajo no se incluyen los tres pacientes con mismo fenotipo y genotipo asociado al gen *TRMT5*.

Tabla 2. Características físicas de los pacientes pediátricos con NH.

Característica	Media (DE) [Rango]
Edad en el reclutamiento, años	12.2 (4.3) [2 a 20]
Talla, m	1.51 (0.20) [1.02 a 1.97]
Peso, kg	49.4 (19.6) [16.0 a 100.0]
Índice de masa corporal (IMC)	20.6 (4.6) [12.8 a 32.2]
Percentil IMC	58.3 (33.0) [0.0 a 99.0]
Índice postura podal, puntuación	-0.1 (3.5) [-12 a 7]
Test de Lunge del tobillo, grados	22.8 (16.8) [0.0 a 50.0]
Puntuación CMTPedS total basal	17.0 (9.2) [1 a 42]

La distribución genética encontrada se muestra a continuación. CMT1A fue el subtipo más frecuente y nuestro segundo subtipo más frecuente fue el portador de mutaciones AD en el gen *GDAP1*.

Tabla 3. Distribución genética de los pacientes pediátricos con NH.

Gen	Número de pacientes de la cohorte (porcentaje)
<i>Duplicación PMP22</i>	37 (37.4)
<i>GDAP1 AD</i>	9 (9.1)
<i>GDAP1 AR</i>	1 (1.0)
<i>GJB1</i>	8 (8.1)
<i>MFN2</i>	3 (3.0)
<i>MPZ</i>	3 (3.0)
<i>HK1</i>	3 (3.0)
<i>BICD2</i>	3 (3.0)
<i>EGR2</i>	2 (2.0)
<i>SH3TC2</i>	2 (2.0)
<i>FGD4</i>	2 (2.0)
<i>NDRG1</i>	1 (1.0)
<i>LITAF</i>	1 (1.0)
<i>DYNC1H1</i>	1 (1.0)
<i>ATP1A1</i>	1 (1.0)
<i>ATL1</i>	1 (1.0)
<i>ARSA</i>	1 (1.0)
Gen desconocido	20 (20.2)

En dicho artículo describimos el fenotipo por separado de las formas desmielinizantes y axonales de NHSM. Se hace especial hincapié en un adolescente portador de una nueva variante probablemente patogénica en el gen *ATP1A1* (c.1645G>A; p.Gly549Arg) que se clasificó como CMT intermedio. Se observó una gran heterogeneidad clínica entre un niño y su padre portadores de la mutación c.1142G>A; p.Arg381His en *EGR2* pues el hijo falleció a los nueve años por complicaciones respiratorias debido a una grave debilidad axial mientras que el padre debutó sólo con pies cavos en la treintena.

RESULTADOS

B. ESTIMACIÓN DE LA SENSIBILIDAD DE CMT PEDS EN NHSM

En la segunda parte de este primer trabajo anexo (Argente-Escrig et al., 2021a) se aborda la sensibilidad de la escala CMT PedS como medida de evaluación en ensayos clínicos determinando si es capaz de detectar cambios a uno o dos años vista en los distintos subtipos de CMT. Evaluamos a 76 niño/as y adolescentes con CMT utilizando la CMT PedS, 62 de los cuales también pudieron completar los 11 ítems de la escala 1 año después y 45 de los cuales completaron a los 2 años. La puntuación total de CMT PedS al inicio osciló entre 1 (leve) a 42 (grave).

Tabla 4. Progresión a lo largo de dos años en función de la puntuación de CMT PedS en los subtipos más frecuentes de CMT.

Subtipo CMT	Puntuación basal [n]	Puntuación al año [n]	Puntuación a los dos años [n]	Diferencia en 1 año	Diferencia en 2 años
Todos casos CMT	17.3±9.7 (1 – 42) [76]	18.1±10.1 (1 – 42) [62]	20.1±10.1 (1 – 38) [45]	1.84±3.7 (95% CI 0.89 – 2.79)**	3.6±4.4 (95% CI 2.3 – 5.0)**
CMT1A	14.9±7.0 (4 – 31) [33]	16.3±7.8 (6 – 34) [29]	19.8±8.3 (5 – 33) [19]	1.7±3.6 (95% CI 0.33 – 3.1)*	4.2±4.3 (95% CI 2.1 – 6.3)**
GDAP1 AD	14.7±11.0 (1 – 33) [9]	15.6±10.9 (1 – 32) [9]	17.0±11.8 (1 – 35) [9]	0.9±3.3 (95% CI -1.6 – 3.4)	2.3±4.2 (95% CI -0.9 – 5.5)

RESULTADOS

<i>GJB1</i>	12.8±6.8 (1 – 23) [8]	14.8±8.3 (2 – 23) [6]	14.5±8.9 (4 – 26) [6]	3.0±4.0 (95% CI -1.2 – 7.2)	2.7±3.9 (95% CI -1.5 – 6.8)
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Los datos son la media ± DE (rango) para el valor inicial y las puntuaciones de seguimiento al año y a los dos años, y la media ± DE (intervalo de confianza del 95 %) para las diferencias: **Cambio significativo desde el valor inicial ($p < 0,0005$); *Cambio significativo desde el inicio ($p < 0,05$).

Hubo una progresión significativa al año y a los dos años de seguimiento para todos los subtipos genéticos de CMT. El empeoramiento de la enfermedad también fue significativo para el subtipo genético más frecuente, CMT1A. Los ítems de la escala con mayor respuesta en un período de un año fueron fuerza de prensión y salto de longitud, esto también fue cierto para el período de dos años de seguimiento.

RESULTADOS

C. CARACTERIZACIÓN FENOTÍPICA Y UTILIDAD DE CMTPEDS EN NHMD

En el segundo trabajo anexo (Argente-Escrig et al, 2021b) se describe el perfil clínico, genético y de discapacidad de una serie de 22 pacientes con NHMD y se estudia la utilidad de la CMTPedS para valorar la discapacidad y la progresión en las neuropatías y neuronopatías hereditarias motoras puras. Dicho trabajo se realizó en colaboración con el grupo de investigación liderado por el Dr. Burns y el Dr. Menezes integrado en *The Children's Hospital at Westmead* (NSW, Australia).

PRESENTACIÓN CLÍNICA

Se incluyeron 22 pacientes menores de 20 años con NHMD. Edad media al primer examen fue a los 9,2 (DE 4,6) años, y la edad de inicio osciló entre el nacimiento y los 10 años, con 14 individuos que presentaban en el primer año de vida. La mayoría se presentaron con deformidad de pie y la función de la mano se vio afectada en 7 individuos, pero la debilidad fue a menudo leve, excepto en 2 pacientes (uno con mutación en *GARS1* y otro en gen desconocido). Signos de neurona motora superior tales como reflejos exaltados, signos de Babinski o espasticidad se observaron en 8 pacientes. 6 pacientes mostraron diferentes grados de afectación cognitiva, de leve a grave discapacidad intelectual.

Se encontraron variantes patogénicas en 9 de 19 familias, proporcionando una tasa de detección del 47 %. El gen más frecuentemente asociado con NHMD en nuestra cohorte fue *BICD2* (n = 7 [4 familias]), seguido de *MFN2* (n = 2), *DYNC1H1* (n = 2), y *GARS1* (n = 1). Las características clínicas (tabla 1) y neurofisiológicas (tabla 2) se hallan detalladas en el trabajo para cada individuo (Argente-Escrig et al, 2021b). Los pacientes con variantes patogénicas en *BICD2* y en *DYNC1H1* tenían un patrón similar en la RM muscular (figura 2).

EVALUACIÓN CON CMTPEDS

Fueron revisadas 32 evaluaciones CMTPedS en 16 niños con NHMd asociado con variantes patogénicas en *GARS* (n = 1), *BICD2* (n = 5), *DYNC1H1* (n = 2), *MFN2* (n = 1), y gen no identificado (n = 7). Doce individuos fueron reevaluados con el CMTPedS a una media de 1,2 (DE 0,2) años. La puntuación total de CMTPedS osciló entre 6 (leve) y 36 (grave). Al inicio del estudio, la edad media era de 13,2 (DE 3,7) años y la enfermedad la gravedad fue moderada en el CMTPedS (media [DE], 18,2 [6.3]) (n = 16). **Los ítems más afectados fueron salto de longitud y equilibrio, mientras que los menos afectados fueron la fuerza de prensión, vibración y pinchazo.** Se observó un efecto suelo para los ítems sensitivos. Todos los elementos correlacionados sustancialmente con al menos otro ítem ($r > 0.3$, $p < 0.05$) a excepción de los ítems de fuerza y sensibilidad del pie. Hubo grandes correlaciones con la puntuación total de CMTPedS ($r > 0.70$, $p < 0.001$) para puntuaciones z de equilibrio y salto de longitud. La consistencia interna para la escala de 11 ítems fue "respetable", con un α de Cronbach de 0.71, sin verse alterada por la eliminación de los ítems de la sensibilidad al pinchazo y de la vibratoria.

Durante 1 año, la puntuación total de CMTPedS se deterioró en promedio 1,5 puntos (DE 3,7 [IC 95 %, -0,5 a 3,5]) o 9 % de la basal. Hubo una gran variabilidad en las tasas de progresión dentro de la cohorte, con individuos con *GARS* y *DYNC1H1* que muestran un empeoramiento significativo, mientras que los individuos con *BICD2* se mantuvieron relativamente estables.

D. FENOTIPADO EN PROFUNDIDAD DE GENOTIPOS CONCRETOS

NHSM ASOCIADO A *FGD4*

El tercer trabajo anexado describe las características clínicas y genéticas de dos hermanos adolescentes portadores de dos variantes no descritas previamente en el gen *FGD4* (CMT4H) que codifica para la proteína frabina (Argente-Escrig et al, 2019).

La mayor de los dos pacientes es una joven de 20 años que permanece completamente asintomática y a la exploración solo se detectó arreflexia aquilea con leve retracción y pie ligeramente cavo. Su hermano (paciente probando) tenía 17 años y participó activamente en los deportes y la exploración era similar a la de su hermana salva por un marcado pie cavo. En los dos pacientes, el ENG mostró una NHSM desmielinizante y la RM muscular de cuerpo entero fue estrictamente normal.

El análisis del panel de genes en el probando identificó dos nuevas variantes candidatas en el gen *FGD4* (NM_139241.2): c.514delG y c.2211dupA. No se detectaron modificaciones a nivel de la transcripción. Se realizó PCR cuantitativa para analizar el ARNm de *FGD4* en el probando que mostró que no había diferencias significativas en la dosis de ARNm de *FGD4* en relación con el control sano y los portadores no afectados. Se predijo que las dos variantes producirían una proteína truncada, la p.Ala172Glnfs*28 (c.514delG) que carece dominios funcionales y el p.Ala738Serfs*5 (c.2211dupA) que los contiene todos.

NHSM ASOCIADO A *TRMT5*

El cuarto y último trabajo anexado tiene por objetivo presentar los datos clínicos, neurofisiológicos, genéticos, imagen cerebral y muscular, biopsia de nervio y músculo y análisis de la actividad del complejo de cadena respiratoria en músculo en tres pacientes con el mismo fenotipo y genotipo asociado a mutaciones en el gen *TRMT5* (Argente-Escrig et al, 2022).

Hemos identificado un haplotipo raro en *TRMT5* ([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]) asociado con desmielinizante polineuropatía en tres familias aparentemente no relacionadas. La neuropatía periférica y la discapacidad intelectual fueron las predominantes características predominantes, los hallazgos adicionales incluyeron: ataxia cerebelosa, signos piramidales y talla baja. Uno de los los pacientes también sufrió crisis febriles complejas las cuales no requirieron medicación. La neuropatía desmielinizante fue predominantemente sensitiva desde el principio, como lo demuestra la ausencia de potenciales evocados sensitivos en todos los estudios neurofisiológicos realizados a los pacientes en la infancia temprana. En la neuroimagen destaca la atrofia cerebelosa global en los tres pacientes. En cuanto al estudio de la biopsia de músculo, se descartó la presencia de rasgos típicamente relacionados con patología mitocondrial (fibras rojo rasgadas, fibras COX negativas). El examen ultraestructural del músculo mostró abundantes cadenas de grandes mitocondrias que ocupan la mayor parte de los espacios intermiofibrilares. En general, el análisis de la actividad del complejo de cadena respiratoria en músculo estaba dentro de los límites normales. El estudio de la biopsia de nervio sural identificó una amplia variedad de anomalías de la mielina, incluida la hipomielinización de las fibras, laminillas de mielina no compactas y plegamiento de mielina focal en microscopía electrónica.

6. DISCUSIÓN

DISCUSIÓN

La presente tesis realiza aportaciones relevantes al conocimiento de las NH en la infancia y la adolescencia, tanto en la descripción de su heterogeneidad clínica y genética, como en la estimación de la sensibilidad de las escalas de discapacidad en el seguimiento la enfermedad.

En primer lugar, confirmamos que nuestro porcentaje de pacientes pediátricos en los que se alcanzaba un diagnóstico genético era muy similar al de los pacientes adultos de la misma región mediterránea (80.6% frente 83.3%) (Sivera et al, 2013) y también bastante próximo a la larga serie pediátrica del *International Neuropathy Consortium* (Cornett et al, 2016). Nuestra serie tenía dos particularidades que la hacían distinta a las previamente publicadas. Una de ellas es que el subtipo de CMT asociado a *MFN2* ocupada el quinto lugar en frecuencia (Argente-Escrig et al, 2021a) mientras que en las otras cohortes pediátricas era el segundo (Cornett et al, 2016; Hoebeke et al, 2018). La otra de las particularidades es que CMT asociada a mutaciones AD en *GDAP1* no estaba presente en otras cohortes pediátricas (Fernández-Ramos et al, 2015; Cornett et al, 2016; Hoebeke et al, 2018) mientras que en la nuestra era el segundo subtipo más frecuente (Argente-Escrig et al, 2021a). Esta alta prevalencia lo más probable es que la serie refleje un efecto fundador de esta mutación: p.Arg120Trp.23 (Sivera et al, 2010).

En segundo lugar, con respecto a las medidas de resultado clínico de CMT, confirmamos que la CMTPedS mide de forma fiable la discapacidad en niños y adolescentes de 3 a 20 años (Burns et al, 2012; Cornett et al, 2016; Argente-Escrig et al, 2021a), y puede detectar el cambio 2 años (Cornett et al, 2017; Argente-Escrig et al, 2021a). Datos de historia natural multicéntricos (n = 187) mostró una progresión significativa de la enfermedad durante 2 años con un ritmo menor para CMT1A comparado con el total de su cohorte (Cornett et al, 2017). A diferencia del anterior estudio, la tasa de progresión fue mayor en nuestro subgrupo CMT1A que en nuestra cohorte general de CMT (Argente-Escrig et al, 2021a), lo que podría reflejar la progresión más lenta en pacientes con *GDAP1* AD (Sivera et al, 2010) que constituyeron el 20% de la cohorte en el seguimiento

de 2 años. Como novedad, **nuestros resultados apuntan a que la CMTpedS es capaz de detectar progresión de la enfermedad al año de seguimiento en los pacientes con CMT1A y en la cohorte entera de CMT** (Argente-Escrig et al, 2021a). Trabajos previos identificaron la fuerza en la dorsiflexión del pie, el equilibrio y el salto de longitud como los ítems que más se modificaban con el tiempo (Cornett et al, 2017). Nuestros hallazgos de que la escala CMTpedS también puede detectar una progresión significativa en el primer año de seguimiento y que la fuerza de prensión también es un ítem sensible puede ayudar a optimizar aún más el diseño de próximos ensayos clínicos.

En tercer lugar, la menor prevalencia de NHMd (Bansagi et al, 2017; Foley et al, 2012), el inicio tardío de algunos subtipos (Harding, 1993) y los desafíos técnicos de realizar estudios sensoriales en neonatos (Yiu y Ryan, 2012a) puede explicar la escasez de literatura sobre NHMd puras en la infancia. En el segundo trabajo indexado (Argente-Escrig et al, 2021b) se presenta una gran cohorte de niño/as y adolescentes con NHMd estudiados extensamente desde el punto de vista clínico, genético y de discapacidad con la escala CMTpedS. La distribución genética en NHMd se ha descrito previamente en solo tres cohortes predominantemente adultas (Dierick et al, 2008; Luigetti et al, 2016; Bansagi et al, 2017). En 2008, 112 pacientes índice con NHMd fueron analizados y en el grupo genéticamente clasificado (n = 17), 8 de los individuos mostraron signos piramidales y sólo 4 pacientes tenían menos de 20 años (Dierick et al, 2008). Las cohortes infantiles de NHMd difieren genéticamente de las cohortes adultas, con solo *BICD2* mostrando una alta frecuencia en ambas cohortes (Argente-Escrig et al, 2021b; Bansagi et al, 2017).

En cuarto lugar, cada vez son más las terapias racionales dirigidas para NHMd (Benoy et al, 2018) por lo que sería muy importante definir escalas capaces de detectar cambios en estos pacientes. Los análisis en esta cohorte sugieren que una escala modelada en el CMTpedS podría ser útil para medir el deterioro funcional en niño/as y adolescentes con dHMN y puede ser útil como medida de resultado en ensayos clínicos (Argente-Escrig et al, 2021b). En nuestra cohorte,

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la enfermedad progresó en el primer año a una tasa de 1.5 ± 3.7 puntos totales en la CMTpedS o un 9% aumento desde la basal, casi el doble del cambio en 1 año en CMT1A (Burns et al, 2012) y equivalente a más de 2 años de seguimiento en CMT1B (Cornett et al, 2017). Hubo una amplia variabilidad en la tasa de progresión dentro de los individuos de la cohorte (Argente-Escrig et al, 2021b), lo que sugiere que la tasa de progresión puede diferir entre subtipos genéticos de NHMd. **El equilibrio fue uno de los ítems más afectados en individuos con NHMd** (Argente-Escrig et al, 2021b) lo que apunta a que el entrenamiento del equilibrio debe convertirse en un importante objetivo de la rehabilitación en las formas motoras y a que la pérdida sensitiva podría no ser la causa principal del equilibrio deficiente en NH. Varios argumentos apoyan la eliminación de los ítems de sensibilidad de una futura escala de discapacidad para NHMd: presentan efecto suelo, explorar la sensibilidad al pinchazo y la vibratoria en niño/as muy pequeños es difícil, la consistencia interna permanece inalterada cuando se excluyen estos ítems y los ítems de sensibilidad fueron omitidos de una escala reciente que mide discapacidad en adultos con CMT (Eichinger et al, 2018).

En quinto lugar, se ha identificado dos nuevas variantes probablemente patogénicas en el gen *FGD4* en dos hermanos con CMT4H (Argente-Escrig et al, 2019). **Ambos diagnosticados con una neuropatía desmielinizante con inicio más tardío y un fenotipo más leve que los descritos previamente.** Desde que se describieron las dos primeras familias con CMT4H (Delague et al, 2007), ésta se ha considerado como una enfermedad desmielinizante de aparición muy temprana con un fenotipo grave. Sin embargo, conforme ha aumentado el número de familias con CMT4H, formas más leves también se han descrito. Eso sí, la característica común a todos los pacientes fue el inicio durante la infancia y la lenta progresión (Delague, 2013) a diferencia de los pacientes presentados en nuestro trabajo (Argente-Escrig et al, 2019). La potencial conservación de los principales dominios funcionales en la frabina de uno de los alelos (p.Ala738Serfs*5) podría explicar parcialmente este fenotipo más leve y de debut más tardío.

Por último, **esta tesis expande el fenotipo de los trastornos mitocondriales causados por mutaciones en el gen *TRMT5* y define una nueva forma de neuropatía desmielinizante recesiva** (Argente-Escrig et al, 2022). La presencia de mutaciones recesivas en el gen *TRMT5* se han en identificado previamente en tres familias (Powell et al, 2015; Tarnopolsky et al, 2017) que comparten la variante patogénica c.312_315del con nuestras familias, una delección que produce un codón de parada prematuro p.Ile105Serfs*Ter4. Sin embargo, la presentación clínica en estos casos anteriores fue muy distinta (Powell et al, 2015; Tarnopolsky et al, 2017), presentando intolerancia al ejercicio, acidosis láctica y evidencia de múltiples deficiencias en la cadena respiratoria mitocondrial del músculo esquelético. Algunos de estos pacientes desarrollaron neuropatías después de décadas de evolución (Haller et al, 1989; Powell et al, 2015; Tarnopolsky et al, 2017), pero nunca fue la neuropatía la característica principal de su síndrome. Nuestros pacientes no mostraron intolerancia al ejercicio o una bioquímica sugestiva de una anomalía OXPHOS (Argente-Escrig et al, 2022). El análisis patológico en los nervios de nuestros pacientes reveló una profunda alteración de la cascada de mielinización desde el inicio del proceso hasta la fase de compactación de laminillas y la regulación del espesor y la forma de la vaina de mielina (Argente-Escrig et al, 2022). Estas características no han sido analizadas a fondo en los otros casos descritos asociados a *TRMT5* (Haller et al, 1989; Powell et al, 2015; Tarnopolsky et al, 2017). Así pues, no podemos concluir si estas anomalías están específicamente asociadas con las mutaciones *TRMT5* portadas por nuestros pacientes o puede ser extrapolable a otros genotipos del mismo gen. A destacar que se observan características compartidas con diversas neuropatías desmielinizantes o dismielinizantes tipo CMT, particularmente aquellas asociadas con fenotipos hipomielinizantes congénitos, como mutaciones puntuales en *PMP22*, *MZP* y otros (Cavallaro et al, 2021) aunque los casos descritos en la presente tesis difieren de muchos de ellos por la ausencia de bulbos de cebolla o reduplicación de la lámina basal (Argente-Escrig et al, 2022). Los tres pacientes aparentemente no relacionados compartían el fenotipo y el

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genotipo pues portan el mismo cambio c.312_315del (p.Ile105Serfs*4) en *TRMT5* y una rara variante c.665 T > C (p.Ile222Thr) (Argente-Escrig et al, 2022). El cambio que altera la pauta de lectura p.Ile105Serfs*4 es relativamente frecuente en la población sana. La variante rara y descrita por primera vez en nuestro trabajo c.665 T > C (p.Ile222Thr) parece prevalecer en nuestra geografía región puesto que la portan estas tres familias aparentemente no relacionadas (Argente-Escrig et al, 2022). El ARN de transferencia (ARNt) metiltransferasa 5 (*TRMT5*) es un gen nuclear (MIM*611023) que codifica una proteína que cataliza la metilación de varios ARNt mitocondriales, una modificación necesaria para mejorar la eficiencia de la traducción (Brulé et al, 2004). Nuestro análisis estructural predice que la sustitución del aminoácido hidrofóbico isoleucina en la posición 222 a un aminoácido polar (treonina) muy probablemente conduce a la desestabilización del dominio D2 y esto podría afectar la unión del ARNt y, en consecuencia, su modificación.

7. CONCLUSIONES

CONCLUSIONES

EN CASTELLANO

De la presente tesis doctoral se pueden extraer las siguientes conclusiones:

1. Esta gran serie pediátrica de NH procedente en su mayoría de un único centro terciario destaca la distribución genética distintiva en esta región mediterránea, con más pacientes con AD *GDAP1* AD que *MFN2* (Argente-Escrig et al, 2021a).
2. Este estudio, además de confirmar la progresión a los dos años de seguimiento, también muestra por primera vez que la CMTPedS es sensible al cambio en el primer año de seguimiento (Argente-Escrig et al, 2021a). Dicho hallazgo puede ayudar a diseñar, de forma más eficiente, ensayos clínicos que evalúen terapias racionales que busquen retrasar o detener la progresión del CMT en la infancia y adolescencia. En estas etapas de la vida, antes de que se produzca más degeneración, es cuando es más útil evaluar este tipo de intervenciones terapéuticas.
3. La NHMd en la infancia es rara, genéticamente heterogénea y, por lo general, lentamente progresiva. La afectación del tracto piramidal y la cognitiva son frecuentes en la NHMd pediátrica (Argente-Escrig et al, 2021b).
4. La escala CMTPedS es una medida de discapacidad sensible en NHMd y muestra progresión durante 1 año (Argente-Escrig et al, 2021b). Nuestro estudio proporciona algunos datos que pueden ayudar a adaptar la CMTpedS a los pacientes con NHMd. Sin embargo, se requieren estudios más amplios para evaluar la tasa de progresión de los subtipos de NHMd y optimizar aún más esta escala como medida de resultado en la población infantil con NHMd para su uso en ensayos clínicos.

CONCLUSIONES

5. Los pacientes portadores de las variantes patogénicas c.514delG (p.Ala172Glnfs*28) y c.2211dupA (Ala738Serfs*5) en el gen *FGD4* debutaron en la adolescencia y mostraron un fenotipo muy leve (Argente-Escrig et al, 2019) a diferencia de lo publicado previamente. La proteína truncada p.Ala738Serfs*5 puede haber conservado parcialmente la actividad de *FGD4* ya que se conservan los principales dominios funcionales. El fenotipado en profundidad y el exhaustivo análisis genético realizado nos ayudan a comprender los mecanismos patogénicos asociados a las diferentes variantes y su influencia en el fenotipo final.
6. Las mutaciones recesivas en el gen *TRMT5* se asocian con una neuropatía sensitivomotora desmielinizante compleja de inicio congénito o infantil (Argente-Escrig et al, 2022). Se debe considerar el cribaje de mutaciones en el gen *TRMT5* cuando un paciente presenta retraso global del desarrollo, neuropatía desmielinizante de predominio sensitivo, signos piramidales y ataxia cerebelosa leve, incluso en ausencia de un perfil bioquímico compatible con una deficiencia de OXPHOS. Las muestras ultraestructurales de músculos y nervios pueden indicar una etiología mitocondrial en casos en que las imágenes histopatológicas de rutina parezcan normales. Dada la ausencia de anomalías mitocondriales estructurales y funcionales prominentes, se necesitan más casos para confirmar nuestros hallazgos.

CONCLUSIONES

EN INGLÉS

The following conclusions can be drawn from this doctoral thesis:

1. This large pediatric series of inherited peripheral neuropathies mostly from a single tertiary center highlights the distinctive genetic distribution in this Mediterranean region, with more AD *GDAP1* than *MFN2* (Argente-Escrig et al, 2021a).
2. This study, in addition to confirming progression at two years of follow-up, also shows, for the first time, that the CMTPedS is sensitive to disease change over one year (Argente-Escrig et al, 2021a). This finding may help design more efficient therapeutic trials of any rational therapy that aims to slow or halt the progression of CMT in children and adolescents. In this period of life, before degeneration occurs, it is most useful to evaluate these types of therapies.
3. Distal hereditary motor neuropathy (dHMN) in childhood are rare, genetically heterogeneous, and usually slowly progressive. Pyramidal tract involvement and cognitive involvement are frequent in pediatric dHMN (Argente-Escrig et al, 2021b).
4. The CMTPedS is a sensitive measure of disability in dHMN and shows progression over one year (Argente-Escrig et al, 2021b). Our study provides some data that may help to adapt the CMTPedS to dHMN patients. However, larger studies are required to evaluate the rate of progression for subtypes of dHMN and to further optimize this scale as an outcome measure in childhood dHMN population for use in clinical trials.
5. The patients carrying the c.514delG (p.Ala172Glnfs*28) and c.2211dupA (Ala738Serfs*5) pathogenic variants in *FGD4* had an adolescent onset and a very mild phenotype (Argente-Escrig et al, 2019) as opposed to previously published cases. The truncated p.Ala738Serfs*5 protein may

have partially conserved FGD4 activity since the main functional domains are retained. The in-depth phenotyping and comprehensive genetic analysis carried out help us understand the pathogenic mechanisms associated with the different mutations and their influence on the final phenotype.

6. *TRMT5* recessive mutations are responsible for a complex demyelinating sensorimotor neuropathy with congenital or infantile onset (Argente-Escrig et al, 2022). Screening for mutations in the *TRMT5* gene should be considered when a patient presents 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 an OXPHOS deficiency. Ultrastructural muscle and nerve specimens might point to a mitochondrial etiology 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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9. ANEXO

A. ARGENTE-ESCRIG H, FRASQUET M, VÁZQUEZ-COSTA JF, ET AL. PEDIATRIC INHERITED PERIPHERAL NEUROPATHY: A PROSPECTIVE STUDY AT A SPANISH REFERRAL CENTER. ANN CLIN TRANS NEUROL. 2021A;8(9):1089-1816.

RESEARCH ARTICLE

Pediatric inherited peripheral neuropathy: a prospective study at a Spanish referral centerHerminia Argente-Escrib ^{1,2,3,4} , Marina Frasset ^{1,2,3,4}, Juan Francisco Vázquez-Costa ^{1,2,3,4}, Elvira Millet-Sancho ^{4,5}, Inmaculada Pitarch ⁶, Miguel Tomás-Vila ⁶, Carmen Espinós ^{4,7}, Vincenzo Lupo ^{4,7} & Teresa Sevilla ^{1,2,3,4,8}¹Neuromuscular & Ataxias Research Group, Instituto de Investigación Sanitaria La Fe, Valencia, Spain²Neuromuscular Diseases Unit, Department of Neurology, Hospital Universitari i Politècnic La Fe, Valencia, Spain³Centre for Biomedical Network Research on Rare Diseases-CIBERER, Valencia, Spain⁴Rare Diseases Joint Unit IIS La Fe – CIPF, Valencia, Spain⁵Department of Clinical Neurophysiology, Hospital Universitari i Politècnic La Fe, Valencia, Spain⁶Department of Pediatrics, Neuropediatrics Unit, Hospital Universitari i Politècnic La Fe, Valencia, Spain⁷Unit of Genetics and Genomics of Neuromuscular and Neurodegenerative Disorders, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain⁸Department of Medicine, University of Valencia School of Medicine, Valencia, Spain**Correspondence**

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Abstract**Background:** Single-center clinical series provide important information on genetic distribution that can guide genetic testing. However, there are few such studies on pediatric populations with inherited peripheral neuropathies (IPNs). **Methods:** Thorough genetic testing was performed on IPN patients under 20 years of age from a geographically well-defined Mediterranean area (Valencian Community, Spain), annually assessed with the Charcot–Marie–Tooth disease Pediatric Scale (CMTPedS). **Results:** From 86 families with IPNs, 99 patients (59 males) were identified, 85 with sensorimotor neuropathy or CMT (2/3 demyelinating form) and 14 with distal hereditary motor neuropathy (dHMN). Genetic diagnosis was achieved in 79.5% families, with a similar mutation detection rate in the demyelinating (88.7%) and axonal (89.5%) forms, significantly higher than in the dHMN families (27.3%). CMT1A was the most common subtype, followed by those carrying heterozygous mutations in either the *GDAP1* or *GJB1* genes. Mutations in 15 other genes were identified, including a new pathogenic variant in the *ATPIA* gene. The CMTPedS detected significant disease progression in all genetic subtypes of CMT, at a rate of 1.84 (± 3.7) over 1 year ($p < 0.0005$, $n = 62$) and a 2-year rate of 3.6 (± 4.4 ; $p < 0.0005$, $n = 45$). Significant disease worsening was also detected for CMT1A over 1 (1.7 ± 3.6 , $p < 0.05$) and 2 years (4.2 ± 4.3 , $p < 0.0005$). **Conclusions:** This study highlights the unique spectrum of IPN gene frequencies among pediatric patients in this specific geographic region, identifying the CMTPedS as a sensitive tool to detect significant disease worsening over 1 year that could help optimize the design of clinical trials.**Introduction**

Inherited peripheral neuropathies (IPNs) are a complex group of diseases with broad phenotypic and genotypic diversity, especially in pediatric populations. Charcot–Marie–Tooth disease (CMT) represents a group of inherited neuropathies with both motor and sensory

involvement, and it is generally classified according to the upper limb motor nerve conduction velocities (MNCVs): demyelinating CMT1 when the MNCV < 38 m/s; or axonal CMT2 if the MNCV > 38 m/s.¹ In some cases, the term “intermediate CMT” is also used when upper limb MNCVs are between 35 m/s and 45 m/s.² The motor-predominant and sensory-predominant ends of the spectrum are

referred to as distal hereditary motor neuropathy (dHMN) and hereditary sensory neuropathy (HSN), respectively.

Advances in molecular genetics in the past 30 years have provided an ever-expanding list of more than 90 genes and loci implicated in IPNs, especially after the discovery of next-generation sequencing (NGS).³ Characterizing large series of patients clinically and genetically is important to obtain information about the natural history and the phenotype-gene relationships in IPNs. In the last 5 years, the multi-center joint effort of the Inherited Neuropathies Consortium (INC) determined the genetic spectrum of CMT in their entire cohort,⁴ as well as in the subgroup aged 3–20 years old.⁵

Nevertheless, analyzing clinical series from single-centers in a uniform and comprehensive manner is still important to shed light on the genetic distributions in these populations, and in geographically well-defined areas, helping to guide genetic testing. The significant delay in diagnosing pediatric IPN populations⁶ and the absence of widespread validated tools to measure disability in this age group until recently⁷ has led to a paucity of large single-center cohorts of children with IPNs^{8,9} relative to adult cohorts.^{2,10–13} Here we report the genetic distribution, phenotypic characterization and natural history over 2 years of disease progression in an extensive series of pediatric IPNs from a single referral center located in a Spanish region with 5,000,000 inhabitants (Valencian Community).

Methods

Patients

This is a longitudinal descriptive study carried out on all patients in whom IPN was the leading feature and who were evaluated prior to the age of 20 at the Neuromuscular Clinic of the Hospital Universitari i Politècnic La Fe (Valencia, Spain), between 2017 and 2020. The diagnosis and classification of IPNs was based on the clinical manifestations, family history, and electrophysiological features.¹⁴ According to sensory nerve conduction studies (NCSs), patients were classified as having CMT (if the sensory NCS was abnormal) or dHMN (if normal). Patients were subclassified as having demyelinating or axonal CMT based on their forearm ulnar MNCV, with a cut-off value of 38 m/s.¹ In patients whose amplitudes of ulnar compound motor action potentials (CMAPs) were reduced >90%, we considered the conduction velocities measured to the flexor carpi ulnaris or the axillary latency.

Nerve conduction studies

NCSs were performed by standard techniques using a Medelec Synergy electromyograph (Mistro, Surrey, UK),

with surface electrode stimulation and recording. Recently published normal values were employed.¹⁵ Electrophysiological recordings were taken from the motor ulnar, median, axillary, peroneal nerves and the sensory (orthodromic) median, (antidromic) sural, and radial nerves. Distal motor latency (DML), MNCV, sensory nerve conduction velocity (SNCV), and the amplitudes (baseline to negative peak) of CMAPs and sensory action potentials (SAPs) were also assessed.

Written informed consent was obtained from the patients themselves or their guardians. This study was approved by the Institutional Review Board of Hospital Universitari i Politècnic La Fe.

Clinical assessments and disease severity

As clinical features of the subjects, we assessed their strength, muscular atrophy, sensory responses, reflexes, and foot deformities, and we conducted a general and a neurological examination. Foot deformity was assessed using the Foot Posture Index,¹⁶ while the ankle joint dorsiflexion was measured by weight bearing using the lunge test and a bubble inclinometer. No achilles retraction was present if the lunge test >35°.¹⁷ The Charcot–Marie–Tooth disease Pediatric Scale (CMTPedS) was administered by the same examiner (HAE) to quantify disease severity in a subset of patients, at baseline, and after 1 and 2 years of disease progression. We were unable to assess the CMTPedS in some patients with intellectual disability or behavioral issues that limited their collaboration. Using the online CMTPedS calculator (<https://www.cmtped.org>), the 11 performance-based items of dexterity, strength, sensation, balance, gait, power, and endurance were converted to categorized scores, ranging from 0 (unaffected) to 4 (severely affected), and these scores were summed to produce a total CMTPedS score ranging from 0 to 44, whereby a higher score indicates greater disease severity. A score of 0–14 is considered mildly affected, a score of 15–29 is defined as moderate, and 30 points or above is considered as severe.⁷

Molecular genetic analysis

Sanger sequencing on an ABI Prism 3730xl (Applied Biosystems, Foster City, CA, USA) was performed to identify the disease-causing mutation in genetically diagnosed families. In all of the probands with demyelinating CMT, the CMT1A duplication was first analyzed by MLPA (Multiplex Ligation-dependent Probe Amplification) on a genetic analyzer ABI Prism 3130xl (Applied Biosystems, Foster City, CA, USA) and using the CMT1 SALSA kit P033-B4 (MRC-Holland, Amsterdam, the Netherlands). If a CMT1A duplication was ruled out,

mutational screening of the *SH3TC2*, *NDRG1*, and *HK1* genes was performed in demyelinating CMT patients of the Roma minority, as described previously.¹⁸ In the remaining undiagnosed index patients, an NGS-targeted custom panel was employed using the Sure Select QXT technology of Agilent technologies (Santa Clara, CA, USA). Any genetically undiagnosed patients after assessment with the gene panel subsequently underwent whole exome sequencing. In all cases, NGS libraries were sequenced using an Illumina system (San Diego, CA, USA). After variant annotation and filtering, variant classification was carried out based on the American College of Medical Genetics (ACMG) guidelines.¹⁹ All pathogenic variants were validated by Sanger sequencing.

Statistical analysis

Statistical analysis was performed with SPSS v. 20.0 (IBM Corp. Armonk, NY). Descriptive data were represented as the mean \pm standard deviation (SD) or as percentages. All data were assessed for normality and the appropriate parametric or nonparametric tests were subsequently used. We used paired *t*-tests to assess the significance of change in the CMTpedS total score between the baseline and 1-year study visit, and between the baseline and the 2-year follow-up visit. An α level <0.05 was defined as statistically significant.

Results

Clinical presentation and genetic distribution

A total of 99 patients (59 males) from 83 families met our inclusion criteria and were considered to have IPN. Nearly half of them (41/99) were descendants of adult patients who had a long-term follow-up at our institution,¹² which enabled us to assess the pediatric patients at early stages of their disease. All held Spanish nationality except for one Chinese citizen, and the majority were Caucasian except for seven Romani descendants, two Afrodescendants, and one Asian. Of the 83 families, 78 (94%) were currently living in the Valencian Community. The average age at disease onset was 3.2 (± 3.0), ranging from birth to 13.8 years of age, and the physical characteristics of each individual are described in Table 1. There were 85 patients from 72 families who presented with CMT (85.9% of the cohort), while 14 from 11 families had dHMN. Initially, 63 patients were classified as demyelinating CMT and 22 as axonal CMT. There were 11 patients that carried specific mutations and that did not undergo neurophysiological examination, and these individuals classified according to the index patient's NCS

results. These 11 patients were either CMT1A ($n = 5$), or they carried specific mutations in the *GDAP1* ($n = 1$), *GJB1* ($n = 4$), or *LITAF* ($n = 1$) genes. Regarding the pattern of inheritance in the entire cohort, this was considered as autosomal dominant (AD) in 55 cases (55.6%), 14 (14.1%) were autosomal recessive (AR), 9 (9.1%) were recessive X-linked, 6 were de novo (6.0%), and 15 (15.2%) were considered sporadic. Consanguinity was detected in 7/99 cases (7.1%). A genetic diagnosis was achieved in 66 of the 83 families (79.5%), with a similar detection rate of mutations in the demyelinating (47/53; 88.7%) and the axonal (17/19; 89.5%) CMT families but significantly higher than in the dHMN families (2/11; 18.2%). Moreover, the genetic distribution in our cohort was compared with the latest published data on pediatric CMT (see Table 2).

Patients with demyelinating CMT (CMT1)

Among these patients, the disease-causing mutation was identified in 55 individuals with the following distribution: 37 carried a PMP22 duplication; 3 mutations in *GJB1*; 3 mutations in *MPZ*; 3 mutations in *HK1*; 2 mutations in *SH3TC2*; 2 mutations in *FGD4*; and one patient carrying mutations in *ARSA*, *LITAF*, *ATP1A1*, *EGR2*, or *NDRG1*. The patient with metachromatic leukodystrophy presented with a demyelinating polyneuropathy at the age of 14 months and a normal brain MRI. At 24 months of age, hyperintensities consistent with leukodystrophy were apparent in brain MRI. We consider the demyelinating neuropathy to be part of metabolic disorder. This patient carried two known mutations in the *ARSA* gene (NM_000487: [c.986C>T; p.Thr329Ile], [c.991G>T; p.Glu331*])^{20,21} and an additional new variant classified as likely pathogenic according to the ACMG criteria

Table 1. Physical description of children with inherited peripheral neuropathies.

Characteristic	Mean (SD) [Range]
Age when recruited, y	12.2 (4.3) [2 to 20]
Height, m	1.51 (0.20) [1.02 to 1.97]
Weight, kg	49.4 (19.6) [16.0 to 100.0]
BMI	20.6 (4.6) [12.8 to 32.2]
BMI percentile	58.3 (33.0) [0.0 to 99.0]
Foot posture index score	-0.1 (3.5) [-12 to 7]
Ankle Lunge test, degrees	22.8 (16.8) [0.0 to 50.0]
CMTpedS total score at baseline	17.0 (9.2) [1 to 42]

The data included here correspond to both the CMT and dHMN phenotype: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CMTpedS, Charcot-Marie-Tooth disease Pediatric Scale; CMT, Charcot-Marie-Tooth disease; dHMN, distal hereditary motor neuropathy.

Table 2. Genetic distribution and comparison to other pediatric series.

Gene	Number of patients (% of sample)			
	Present work (n = 99)	Cornett et al. (n = 520)	Hoebeke et al. (n = 75)	Fernandez-Ramos et al. (n = 36)
<i>dupPMP22</i>	37 (37.4)	252 (48.5)	46 (61.3)	16 (44.4)
<i>GDAP1</i> AD	9 (9.1)			
<i>GDAP1</i> AR	1 (1.0)	3 (0.6)	1 (1.3)	
<i>GJB1</i>	8 (8.1)	10 (1.9)	2 (2.6)	1 (2.7)
<i>MFN2</i>	3 (3.0)	31 (6.0)	11 (14.7)	1 (2.7)
<i>MPZ</i>	3 (3.0)	15 (2.9)	1 (1.3)	
<i>HK1</i>	3 (3.0)		1 (1.3)	
<i>BICD2</i>	3 (3.0)			
<i>EGR2</i>	2 (2.0)			
<i>SH3TC2</i>	2 (2.0)	13 (2.5)		
<i>FGD4</i>	2 (2.0)	1 (0.2)		
<i>NDRG1</i>	1 (1.0)		1 (1.3)	1 (2.7)
<i>LITAF</i>	1 (1.0)	1 (0.2)		
<i>DYNC1H1</i>	1 (1.0)			
<i>ATP1A1</i>	1 (1.0)			
<i>ATL1</i>	1 (1.0)			
<i>ARSA</i>	1 (1.0)			
<i>PMP22</i> point		9 (1.7)		1 (2.7)
<i>TRPV4</i>		1 (0.2)		1 (2.7)
<i>GARS1</i>		4 (0.8)		
<i>NEFL</i>		3 (0.6)		
<i>MTMR2</i>		2 (0.4)		
<i>PRX</i>		1 (0.2)	1 (1.3)	
<i>FIG4</i>		4 (0.8)		
<i>CMTX3</i>		6 (1.2)		
<i>locus</i>				
<i>HINT1</i>				2 (5.6)
<i>LMNA</i>			1 (1.3)	
<i>GAN</i>			1 (1.3)	
<i>YARS</i>			1 (1.3)	
<i>GDAP1</i> and <i>MFN2</i> ¹			2 (2.6)	
Unidentified gene	20 (20.2)	127 (24.4)	6 (8.0)	13 (36.1)

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; CMTX3 locus, large DNA interchromosomal insertion in Xq27.1.

¹Patients who carried combined heterozygous mutations in both *GDAP1* and *MFN2*; *dupPMP22*, CMT1A duplication; *PMP22*point, point mutation in the *PMP22* gene.

(NM_000487: c.902G>C; p.Arg301Pro). We identified a new variant in the *ATP1A1* gene (NM_001160233: c.1645G>A; p.Gly549Arg) in an adolescent whose CMT was classified as intermediate (ulnaris MNCV 34.4 m/s, CMAP amplitude 12.3 mV). His affected father also carried the same variant and would have also been classified as having an intermediate form of CMT (ulnaris MNCV 41.3 m/s, CMAP amplitude 9.0 mV). Following ACMG criteria, the novel variant in *ATP1A1* was classified as

likely pathogenic based on its absence in control databases (gnomAD, ExAC, 1000G), its co-segregation with the disease in three generations of affected family members and a computationally predicted deleterious effect. We observed strong clinical heterogeneity between a proband and his father who both carried a heterozygous mutation in the *EGR2* gene (NM_000399: c.1142G>A; p.Arg381His). The son was diagnosed with CMT1 aged 4 (ulnaris MNCV <10 m/s), which spread to the upper limbs at 5, and he used a wheelchair and developed a diaphragmatic weakness at 7, dying at the age of 9 due to a bilateral pneumonia. His father was diagnosed at age 39 (ulnaris MNCV around 20 m/s) and his sole symptom since his early thirties had been high arched feet.

Patients with axonal CMT (CMT2)

From a total of 22 patients with axonal CMT, we identified *GDAP1* mutations in 10, the most frequent cause of axonal CMT. Of these, nine patients harbored the AD *GDAP1* mutation (NM_018972: c.358C>T; p.Arg120Trp) and the remaining patient carried an homozygous mutation in *GDAP1* (NM_018972: c.844C>T; p.Arg282Cys). In terms of frequency, *GDAP1* was followed by various mutations in *GJB1* ($n = 5$) and *MFN2* ($n = 3$). Most carriers of the *GDAP1* p.Arg120Trp mutation (5/9) had a moderate CMTpedS score, despite the wide a range in clinical severity with two 12- and 13-year-old siblings scoring 29 and 33 in the CMTpedS, respectively (see Fig. 1). The only patient with a homozygous *GDAP1* mutation scored 22 in the CMTpedS at age 16 (within the moderate range). A heterozygous mutation in *EGR2* (NM_000399: c.1226G>A; p.Arg409Gln) and a de novo mutation in the *ATL1* gene (NM_015915: c.1223T>C; p.Met408Thr) were responsible for the disease in two other individuals. The one with the *ATL1* mutation presented with severe, infant onset axonal CMT, with spasticity and profound intellectual disability.

Patients with dHMN

From a total of 14 patients with dHMN, we identified disease-causing variants in 5 of them: three siblings carried the c.320C>T (p.Ser107Leu) mutation in *BICD2* and one individual harbored a de novo mutation in *DYNC1H1* (c.917A>G; p.His306Arg). Detailed clinical and genetic descriptions on these set of patients have already been published.²²

Natural history

We assessed 76 children and adolescents with CMT using the CMTpedS, 62 of whom were also able to complete all

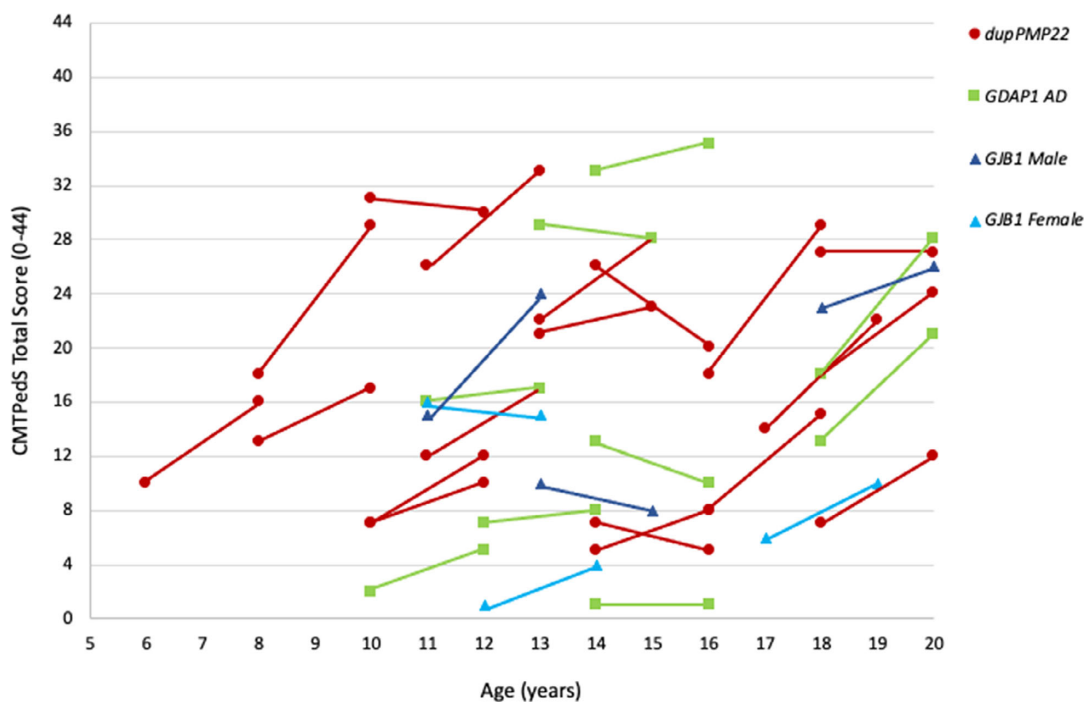


Figure 1. Progression of the total CMTPedS score in relation to the most common CMT genetic subtypes. Each slope represents a patient's change in the CMTPedS Total score over 2 years. Abbreviations: CMTPedS, Charcot-Marie-Tooth disease Pediatric Scale (mild [0–14], moderate [15–29], and severe [30–44]); CMT, Charcot-Marie-Tooth disease; *dupPMP22*, CMT1A duplication; AD, autosomal dominant.

11 items of the scale 1-year later and 45 of whom completed the CMTPedS over a 2-year period. None underwent surgical correction between these assessments. The distribution of the genes affected in the CMT patients assessed with the CMTPedS was: *PMP22dup* ($n = 32$), *GDAP1* ($n = 10$), *GJB1* ($n = 8$), *MPZ* ($n = 3$), *HK1* ($n = 3$), *FGD4* ($n = 2$), *MFN2* ($n = 2$), *EGR2* ($n = 2$), *ATP1A1* ($n = 1$), *LITAF* ($n = 1$), *NDRG1* ($n = 1$), and an unidentified gene ($n = 11$).

The CMTPedS total score at baseline ranged from 1 (mild) to 42 (severe). There was significant progression over 1 year at a rate of $1.84 (\pm 3.7)$ and over 2 years at a rate of $3.6 (\pm 4.4)$ for all the genetic subtypes of CMT ($p < 0.0005$). Disease worsening was also significant for the most frequent genetic subtype, CMT1A (Table 3). The progression of the total CMTPedS score over 2 years is represented in figure 1 for the most common genotypes. There were insufficient numbers of children and adolescents to evaluate the disease progression associated with other genetic subtypes, such as CMT1B, CMT2A, and CMT4G. The most responsive items in a 1-year period ($n = 62$) were grip strength (z -score change of -0.6 ,

95% CI -1.0 to -0.25 , $p = 0.001$) and long jump (z -score change of -0.32 , 95% CI -0.6 to -0.06 , $p = 0.017$). This was also true for the 2-year period ($n = 45$) with grip strength (z -score change of -0.6 , 95% CI -0.92 to -0.31 , $p < 0.0001$) and long jump (m -score change of -0.46 , 95% CI -0.83 to -0.09 , $p = 0.017$) showing a significant change relative to the baseline.

Discussion

In the present series of IPN patients, 80.6% of the individuals obtained a genetic diagnosis ($n = 99$), a very similar value to the 83.3% described in the adult series from the same Western Mediterranean area ($n = 438$)¹² and close to the 75% observed in a large pediatric series reported by the International INC ($n = 520$).⁵ The success rates for genetic diagnosis in CMT1 and CMT2 families were comparable in our cohort (88.7% and 89.5%, respectively), although other studies carried out mainly on adults reported higher genetic hit rates for CMT1 than CMT2 (approximately 90–95% vs. 40–60%).^{2,11,12} Our high rate of mutation detection among CMT2 patients

Table 3. Disability progression over 2 years according to the CMTPedS Total score in the most frequent CMT genetic subtypes.

CMT type	Baseline score [n]	1-year FUP score [n]	2-year FUP score [n]	Difference over a year	Difference over 2 years
All CMT cases	17.3 ± 9.7 (1–42) [76]	18.1 ± 10.1 (1–42) [62]	20.1 ± 10.1 (1–38) [45]	1.84 ± 3.7 (95% CI 0.89–2.79)**	3.6 ± 4.4 (95% CI 2.3–5.0)**
CMT1A	14.9 ± 7.0 (4–31) [33]	16.3 ± 7.8 (6–34) [29]	19.8 ± 8.3 (5–33) [19]	1.7 ± 3.6 (95% CI 0.33–3.1)*	4.2 ± 4.3 (95% CI 2.1–6.3)**
<i>GDAP1</i> AD	14.7 ± 11.0 (1–33) [9]	15.6 ± 10.9 (1–32) [9]	17.0 ± 11.8 (1–35) [9]	0.9 ± 3.3 (95% CI –1.6–3.4)	2.3 ± 4.2 (95% CI –0.9–5.5)
<i>GJB1</i>	12.8 ± 6.8 (1–23) [8]	14.8 ± 8.3 (2–23) [6]	14.5 ± 8.9 (4–26) [6]	3.0 ± 4.0 (95% CI –1.2–7.2)	2.7 ± 3.9 (95% CI –1.5–6.8)

The data are the mean ± SD (range) for baseline, and the 1 and 2 year follow-up scores, and the mean ± SD (95% Confidence Interval) for the differences: **Significant change from baseline ($p < 0.0005$); *Significant change from baseline ($p < 0.05$). Abbreviations: CMTPedS, Charcot-Marie-Tooth disease Pediatric Scale; CMT, Charcot-Marie-Tooth disease; FUP, follow-up; AD, autosomal dominant.

may be related to the large proportion of patients carrying heterozygous mutations in the *GDAP1* and *GJB1* genes. We found CMT1A to be the most common genetic subtype of CMT, in accordance with most pediatric series.^{5,8,9} Our second most frequent subtype was that carrying AD mutations in the *GDAP1* gene. This genetic subtype was not present in other pediatric cohorts^{5,8,9} and the high prevalence of AD *GDAP1* mutations in our series most likely reflects a founder effect of this specific mutation: p.Arg120Trp.²³ The two most severely affected patients with dominant *GDAP1* mutations were children of a clinically asymptomatic father. Subsequent studies showed that they also carried a mutation in the *JPH1* gene inherited from their healthy mother.²⁴ *JPH1* and *GDAP1* play a role in calcium homeostasis, and the combination *JPH1* p.Arg213Pro and *GDAP1* p.Arg120Trp leads to a reduced store-operated calcium entry activity.²⁴ The patient in our cohort with the homozygous p.Arg282Cys mutation in the *GDAP1* gene had a milder phenotype than the majority of patients harboring AR inherited mutations in this gene.²⁵ It is interesting to note that the only family described with the same mutation also had a fairly benign course.²⁶ Our cohort had few cases of *MFN2* neuropathy (3%), like other Spanish series,^{9,12} yet this contrasted with other pediatric cohorts from the INC⁵ and in France,⁸ in which *MFN2* mutations were the second most frequent.

Here, we described a new pathogenic variant in the *ATPIA1* gene and its clinical correlations. Mutations in *ATPIA1*, which encodes the alpha-1 subunit of the Na²⁺/K⁺-ATPase, were recently identified as a cause of AD axonal CMT in seven unrelated families.²⁷ Subsequently, heterozygous de novo mutations in *ATPIA1* were reported in individuals with renal hypomagnesemia, refractory seizures, and intellectual disability,²⁸ and in a child with spastic paraplegia and intellectual disability with normal nerve conduction.²⁹ Our patient with the new AD variant in the *ATPIA1* gene (p.Gly549Arg) had

no pyramidal signs or intellectual disability at examination, and his CMT could be classified as intermediate, in accordance with previous observations from two Chinese families.³⁰ The child carrying the p.Arg381His variant in the *EGR2* gene suffered from a very severe demyelinating phenotype but did not show signs of cranial neuropathy. This same *EGR2* mutation was previously associated with severe demyelinating CMT with cranial nerve involvement.³¹ The severe disability displayed by our proband carrying the p.Arg381His *EGR2* mutation contrasted with the mild presentation of his father. This might suggest that this mutation is not the sole alteration responsible for the individual's phenotype and that genetic modifiers may influence the clinical heterogeneity.³² In patients with dHMN, mutations were only identified in genes like *BICD2* and *DYNC1H1* that are responsible for lower extremity dominant spinal muscular atrophy (SMALED). Our patients fit this phenotype. Indeed, we did not find any individual's carrying mutations in *HSPB1*, even though this is a gene very frequently affected in dHMN patients, confirming the late onset of mutations in this gene.³³

Regarding clinical outcome measures of CMT, CMTPedS reliably measures disability in children and adolescents from 3 to 20 years of age, and it can detect change over 2 years.⁷ Multicenter natural history data ($n = 187$) showed significant disease progression over 2 years at a rate of 2.4 (±4.9) for all genetic subtypes of CMT ($p < 0.001$) and at a rate of 1.8 (±4.2) for CMT1A ($p < 0.001$).³⁴ We also found the scale to be sensitive to change over 2 years in our single-center cohort. However, the rate of progression was higher in our CMT1A subgroup (4.2 ± 4.3, $p < 0.0005$, $n = 19$) than in our overall CMT cohort (3.6 ± 4.4, $p < 0.0005$, $n = 45$), which might reflect the effect of the slower progression in patients with AD *GDAP1* mutations (2.3 ± 4.2, $n = 9$) as these latter patients were 20% of the cohort at the 2-year follow-up. CMT caused by the AD p.Arg120Trp mutation in the *GDAP1* gene is

thought to be particularly mild and with slow progression.²³ The progression of CMT1A, AD *GDAP1*, and *GJB1* was fairly variable over 2 years in each individual, perhaps influenced by growth as suggested previously.⁵ Our results support the capacity of CMTPedS to detect disease progression over 1 year, all CMT and CMT1A patients displaying significant progression. Foot dorsiflexion strength, balance and the length of jumping were previously found to be the most responsive items.³⁴ In our cohort, the most responsive items were grip strength and the length of jumping. To optimize CMT clinical trials, the INC estimated that young (3–8 years) and mildly affected (CMTPedS score <15) CMT1A cases were the most responsive in terms of the CMTPedS over 2 years of disease progression.³⁵ The observation that the CMTPedS can also detect significant progression over 1 year and that grip strength is also a responsive item may help further optimize the design of forthcoming clinical trials.

Our prospective study is not without limitations. Most genetic subtypes had three patients or less, precluding a natural history analysis. As these patients were recruited from a neuromuscular clinic, patients in whom peripheral neuropathy was not the cardinal feature may be under-represented.

Conclusions

This large pediatric series of IPN from a single tertiary center highlights the distinctive genetic distribution in this Mediterranean region, with more AD *GDAP1* patients than *MFN2*. Evaluation in children and adolescents may be key to highlight the role of modifier genes (e.g., *JPH1* as a modifier of *GDAP1*-associated CMT), since at their young age they have not been exposed to external factors like medical co-morbidities, drugs or other toxins. This study also shows the CMTPedS to be sensitive to disease change over 1 year, which may help design more efficient therapeutic trials in children of any rational therapy that aims to slow or halt the progression of CMT.

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Author Contributions

HAE and TS designed the study. HAE assessed the patients, conducted the statistical analysis, and elaborated the first draft. MF, JFVC, EMS, IP, MT, and TS provided detailed patient information and critically revised the manuscript. CE and VL performed the genetic studies and reviewed the article for important intellectual content.

Conflict of Interest

All authors read and approved the final manuscript and have no conflicts of interest to disclose.

Ethics Approval and Consent to Participate

Written informed consent was obtained from all the patients or their guardians/parents, and the protocols were approved by the Institutional Review Board and Ethics Committee at the Hospital Universitari i Politècnic La Fe (Valencia, Spain).

Patient Consent for Publication

Not applicable.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ARTICLE

Clinical, Genetic, and Disability Profile of Pediatric Distal Hereditary Motor Neuropathy

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Abstract

Objective

To describe the clinical, genetic, and disability profile of pediatric distal hereditary motor neuropathy (dHMN) and to determine the utility of an outcome measure validated for children with Charcot-Marie-Tooth disease (CMT) in assessing disability in this cohort.

Methods

We reviewed the clinical, neurophysiologic, and disability data on individuals with dHMN, evaluated before the age of 20 years, at 2 tertiary neuromuscular clinics in Australia and Spain. Disability was assessed annually with the CMT Pediatric Scale (CMTPeS) in a subset of individuals.

Results

Twenty-two children (13 female) from 19 families were included. Fourteen individuals were symptomatic in the first year of life. Intellectual disability was present in 6 individuals; upper motor neuron signs were seen in 8. Pathogenic variants were found in 9 families, more frequently in *BICD2* (*BICD2-4*, *DYNC1H1-2*, *MFN2-2*, *GARS-1*). A novel pathogenic variant in the *GARS* gene was detected and characterized phenotypically. Disability was moderate on the CMTPeS (mean [SD] 18.2 [6.3], n = 16), with balance and long jump being the most affected and sensation items and grip strength the least affected. Over 1 year, the CMTPeS total score deteriorated, on average 1.5 points (SD 3.7) or 9% (n = 12), with significant variability in the rate of progression within the cohort.

Conclusions

The genetic profile of pediatric dHMN is different from that identified in adult cohorts. This study has identified distinct functional limitations for the CMTPeS in children and adolescents with dHMN.

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Glossary

CHW = The Children's Hospital at Westmead; **CMAP** = compound motor unit action potentials; **CMT** = Charcot-Marie-Tooth disease; **CMTPedS** = CMT Pediatric Scale; **dHMN** = distal hereditary motor neuropathies; **HDAC** = histone deacetylase; **HLF** = Hospital Universitari i Politècnic La Fe; **NCS** = nerve conduction studies; **SMA-LED** = spinal muscular atrophy, lower extremity dominant; **UMN** = upper motor neuron.

The distal hereditary motor neuropathies (dHMN) are a genetically heterogeneous group of inherited neuropathies with slow and symmetric degeneration of the distal lower motor neuron.¹ While sometimes classified under the broad umbrella of Charcot-Marie-Tooth disease (CMT), sensory symptoms are absent or mild in dHMN,² with absence of sensory nerve abnormalities on nerve conduction studies (NCS),³ contrasting with more prominent sensory loss, and both sensory and motor nerve abnormalities on NCS in CMT. The term distal spinal muscular atrophy, reflecting the earlier belief that the pathology existed primarily within the anterior horn cell,⁴ is used interchangeably with dHMN.¹ Motor-predominant syndromes with predominantly proximal involvement are not included within the dHMN classification,¹ although genetic overlap is again recognized between these 2 conditions.⁵ Spinal muscular atrophy, lower extremity dominant (SMA-LED), although it presents as non-length-dependent weakness, is included in dHMN because it is predominant in the lower limbs.^{6,7} A number of genes have recently been identified as specifically causing the dHMN phenotype in adults, assisting with targeting genetic testing and designing custom genetic panels.⁸⁻¹⁰ Isolated motor neuropathy is rare in childhood inherited neuropathy, leading to a paucity of literature on dHMN in this age group. Characterizing the clinical phenotype and genetic heterogeneity, disease burden, and distinct disability profile of dHMN, especially in children, is important for accurate diagnosis and to design meaningful outcome measures for future clinical trials. The clinical and genetic overlap between dHMN and axonal CMT (CMT2) suggests that a well-validated scale for CMT such as the CMT Pediatric Scale (CMTPedS),¹¹ may have applicability in children with dHMN.

Methods

Patients

This study was a retrospective study with data included from the medical records of individuals diagnosed with dHMN at 2 tertiary neuromuscular clinics, The Children's Hospital at Westmead (CHW), NSW, Australia, and the Hospital Universitari i Politècnic La Fe (HLF), Valencia, Spain. Both neuromuscular clinics have detailed databases of children and adolescents assessed in the clinics since 2009, and all patients attending the clinics have demographic, clinical, neurophysiological, and CMTPedS data collected prospectively as part of long-term ethics-approved studies run by the NIH-funded Inherited Neuropathies Consortium. Eligibility criteria were based on clinical and electrophysiological features. Data were

included from patients who were first evaluated in clinic before the age of 20 years with a diagnosis of dHMN based on presentation with all of the following: chronic slowly progressive distal weakness or foot deformity, normal sensory examination, preserved sensory nerve amplitudes, and either reduced compound motor unit action potentials (CMAP) or neurogenic changes on needle EMG.

Minor sensory symptoms such as transient paresthesia did not preclude patient inclusion. Individuals with predominantly proximal weakness or those with NCS showing sensory abnormalities were excluded. Acquired causes were excluded by a history compatible with dHMN (slow progression with disease onset at an early age), targeted metabolic testing, and absence of risk factors context that might suggest an acquired neuropathy (acute onset, critical illness, drug toxicity, or another systemic disorder). No clinical assessments or genetic testing was done prospectively as part of this current study.

Standard Protocol Approvals, Registrations, and Patient Consents

The data used in this study were collected prospectively as part of larger studies approved by the respective local research ethics committees (HREC/16/SCHN/31 at CHW and 2017/0351 at HLF). All patients reported here (or their parents/guardians) gave written informed consent before beginning the study, including consent for publication.

Disability Assessments

In a subset of 16 patients, evaluated since 2010, we included data on baseline disability and disease progression as assessed on the CMTPedS. The CMTPedS comprises 11 performance-based items related to dexterity, strength, sensation, balance, gait, power, and endurance.¹¹ Raw scores are converted to age- and sex-matched normative reference values from the 1000 Norms Project^{12,13} to obtain z scores. The z scores are categorized to a Likert scale ranging from 0 (unaffected) to 4 (severely affected). A category score of 0 represents a z score within 1 SD from the normative reference value mean, and a score of 1, 2, or 3 indicates a z score of 1 to 2, 2 to 3, or 3 to 4 SDs below normative values, respectively, and a score of 4 represents >4 SDs below normal. These categorized scores are summed to produce a CMTPedS total score ranging from 0 to 44 (the highest score indicating the most severe phenotype). A score of 0 to 14 is considered mildly affected, whereas moderate is defined as 15 to 29 and severe as ≥30 points.

Electrophysiology

All electrophysiologic examinations had been performed by experienced neurophysiologists at one of the aforementioned institutions. Motor and sensory NCS were performed, and qualitative and quantitative analyses of motor unit potentials and spontaneous activity were assessed on EMG. Only 1 child did not undergo electrophysiologic study, although NCS of an affected sibling with the same genetic variant was already available for review.

Molecular Genetic Analysis

Probands had been tested on a customized targeted next generation sequencing panel using SureSelect_AllExonV5 kit (at CHW) and SureSelectQXT (at HLF) technology for Illumina (Agilent, Santa Clara, CA). Those patients still undiagnosed underwent whole-exome sequencing. Whole-exome sequencing methodology that was applied to individuals evaluated at CHW has been previously reported.¹⁴ At HLF, the SureSelect Human All Exon V5 kit (Agilent Technologies) was used to perform DNA fragmentation and exome enrichment following the manufacturer's instructions. The captured libraries were sequenced on HiSeq4000 (Illumina) in paired-end mode with a read length of 2×101 bp to generate minimum median raw target coverage of $125\times$. Image analysis, base calling, and quality scoring of the run were processed with the manufacturer's software Real Time Analysis (version 2.7.7) and followed by generation of FASTQ sequence files. Sequence alignment and variant calling were performed against a version of the reference human genome (GRCh37) called hs37d5 and followed an already described bioinformatics pipeline in which variant annotation and filtration are also specified.¹⁵ Variants in a known neuropathy-associated gene were prioritized. Variant classification followed the criteria of the American College of Medical Genetics and Genomics. Novel variants were considered pathogenic if they were identified in a known neuropathy-associated gene, were absent in the control databases (ExAC, gnomAD, and National Center for Biotechnology Information), were predicted to be deleterious (highly conserved, altered protein function or structure in silico), and arose de novo in patients without a family history of inherited neuropathy. Putative pathogenic variants were confirmed by Sanger sequencing using standard methods and were tested for segregation. In silico analyses of variants were performed with PROVEAN, SIFT, Polyphen2, and MutationTaster.

Statistical Methods

Data were analyzed with SPSS version 22.0 (IBM Corp, Armonk, NY) and expressed as mean and SD for quantitative variables and absolute and relative frequencies for qualitative variables. We analyzed the following aspects on the CMTPedS assessments results at baseline: interitem correlations for both scores and z scores (determined by Pearson correlation coefficient), correlation of z score of individual items with CMTPedS total score (using Pearson), and internal consistency (calculated with Cronbach α). The significance of change in CMTPedS Total score over a 1-year

period was assessed with repeated-measures analysis of covariance with time to follow-up as a covariate, and the p value was Bonferroni adjusted. Differences were considered significant at a probability level of $p < 0.05$.

Data Availability

Anonymized data used and analyzed for this report will be shared on reasonable request.

Results

Clinical Presentation

We identified 22 children (13 female) from 19 families with dHMN. Two individuals who met clinical criteria were excluded due to the lack of neurophysiologic studies. A phenotypic description of 7 individuals with *BICD2* included in this cohort has been previously published.^{16,17} Mean age at first examination was 9.2 (SD 4.6) years, and age at onset ranged from birth to 10 years, with 14 individuals presenting in the first year of life. Most presented with foot deformity ($n = 11$), while others presented with generalized hypotonia ($n = 2$), distal lower limb contractures ($n = 4$), or delayed walking ($n = 4$). Hand function was affected in 7 individuals, but weakness was often mild except for 2 patients (*GARS* $n = 1$, undiagnosed $n = 1$). Upper motor neuron (UMN) signs such as brisk reflexes, Babinski signs, or spasticity were observed in 8 patients. Cranial nerve abnormalities or bulbar weakness was not identified in our cohort. Six patients showed different degrees of cognitive involvement, from mild to severe intellectual disability. Generally, disease course was assessed as stable by treating neurologist, parents, and patients themselves.

Pathogenic variants were found in 9 of 19 families, providing a 47% overall detection rate. The inheritance pattern was autosomal dominant in 42% (8 of 19 families). A history of a similarly affected family member existed in 7 of 9 of the genetically classified families but in only 1 of 10 families of the genetically undiagnosed. The gene most frequently associated with dHMN in our cohort was *BICD2* ($n = 7$ [4 families], figure 1, A and B), followed by *MFN2* ($n = 2$), *DYNC1H1* ($n = 2$, figure 1C), and *GARS* ($n = 1$, figure 1I). The clinical features of the cohort are detailed in table 1. Whole-body 3T muscle MRIs were performed in 15 individuals, and those with pathogenic variants in *BICD2* ($n = 5$, age range 9–15 years) and in *DYNC1H1* ($n = 1$) conformed to a common pattern as shown in figure 2.

Neurophysiology

Neurophysiologic studies were available for review in 21 children (table 2). Unlike the rest of the genetically classified cohort, the motor NCS of the individual carrying a pathogenic variant in *GARS* (CHW-1) showed a non-length-dependent motor axonal neuropathy with early distal upper extremity involvement because tibial and peroneal CMAPs were still recordable while the median CMAP had its amplitude <0.5

Figure 1 Clinical Heterogeneity of Pediatric dHMN

(A–F) Lower limb-predominant distal hereditary motor neuropathy (dHMN) and (G–I) upper limb-predominant dHMN. Spinal muscular atrophy, lower extremity dominant phenotype is represented here by (A and B) 2 siblings with a pathogenic variant in *BICD2* at 16 years (HLF-1, A) and 9 years (HLF-2, B) years of age, respectively, and (C) an individual with a pathogenic variant in *DYNC1H1* 17 years of age (HLF-6). (D–F) Two unrelated children at 11 (HLF-8, D) and 8 (HLF-9, E and F) years of age, respectively, presenting with a genetically unclassified nonprogressive dHMN. Notice the absence of hand and upper leg atrophy compared to the severe atrophy below the knee and marked foot deformity that was identified on antenatal ultrasound in both patients. (G and H) A 16-year old patient (HLF-7) with a genetically unclassified upper limb-predominant dHMN. (I) An individual at 8 years of age (CHW-1) carrying a pathogenic variant in *GARS*.

mV. Conduction blocks were also detected in the *GARS* patient (not shown in the table).

CMTPedS Assessment

We reviewed 32 CMTPedS assessments in 16 children (9 females) who had dHMN associated with pathogenic variants in *GARS* ($n = 1$), *BICD2* ($n = 5$), *DYNC1H1* ($n = 2$), *MFN2* ($n = 1$), and unidentified gene ($n = 7$). Twelve individuals were reassessed with the CMTPedS at a mean of 1.2 (SD 0.2) years. The CMTPedS total score ranged from 6 (mild) to 36 (severe), and the progression over time for each individual with a genetic diagnosis is presented in figure 3.

At baseline, mean age was 13.2 (SD 3.7) years, and disease severity was moderate on the CMTPedS (mean [SD], 18.2 [6.3]) ($n = 16$). The most affected items were long jump and balance with 12 and 11 individuals, respectively, scoring >3 SDs below normal (figure 4), while the least affected were grip strength, vibration, and pinprick, with only 1 individual beyond 2 SD of normal (figure 4). A floor effect was observed for the sensation items with 15 of 16 individuals graded as normal

at baseline (figures 4) and 11 of 12 patients also scored 0 (normal) at their 1-year follow-up visit. Grip strength scores were >0 (not affected) at baseline and increased 1 point over 1 year only in the 2 patients with upper limb-predominant dHMN (1-*GARS* and 1-undiagnosed). All items correlated substantially with at least 1 other item ($r > 0.3$, $p < 0.05$) except for foot strength and sensation items. Plantarflexion and dorsiflexion strength did correlate moderately with at least 1 other item, but these correlations did not reach statistical significance. There were large correlations with CMTPedS total score ($r > 0.70$, $p < 0.001$) for z scores of balance and long jump (table 3). Internal consistency for the 11-item scale was “respectable,” with a Cronbach α of 0.71, and the removal of both pinprick and vibration did not alter this (Cronbach $\alpha = 0.73$).

Over 1 year, the CMTPedS total score deteriorated on average 1.5 points (SD 3.7 [95% CI, -0.5 to 3.5]) or 9% from baseline ($n = 12$). There was significant variability in the rates of progression within the cohort, with individuals with *GARS* and *DYNC1H1* showing a significant increase (worsening) of

Table 1 Clinical Features of Children With dHMN

Gene (n) patient ID	Pathogenic variant (nucleotide c; aa c)/ inheritance	Age at onset/age at first examination	Phenotypic characteristics and baseline CMTPedS
GARS (1) CHW-1	c.613G>C ^a ; p.(Asp205His) ^a / <i>dnAD</i>	16 mo/3 y	Severe UL atrophy and weakness with hand function affected early (2 y). AFOs (3 y). CMTPedS 28/44 (6 y).
BICD2 (7) CHW-2 CHW-3 HLF-1 HLF-2 HLF-3 HLF-4 HLF-5	c.320C>T; p.(Ser107Leu) (2 families)/AD	Birth–8 y/1–14 y	Moderate SMA-LED (2 siblings). Hypotonia and contractures at birth. OSA (4 y). Wheelchair (6–9 y). 1/2 mID. CMTPedS 17–22/44 (11–14 y). Mild SMA-LED (3 siblings). 1/3 hip dysplasia. CMTPedS 6–17/44 (8–15 y).
DYNC1H1 (2) CHW-5 HLF-6	c.751C>T; p.(Arg251Cys)/AD	Birth/4 y	Severe SMA-LED. Congenital contractures and delayed walking. mID. Wheelchair (11 y). CMTPedS 29/44 (18 y).
MFN2 (2) CHW-6 CHW-8	c.917A>G; p.(His306Arg)/ <i>dnAD</i>	<1 y/16 y	Mild SMA-LED. Foot deformity (<1 y). mID. Normal brain MRI. CMTPedS 13/44 (16 y).
CHW-10	c.280C>G; p.(Arg94Gly)/AD	5 y/7 y	Severe dHMN with hand atrophy (7 y). AFOs and wheelchair/walker (8 y).
CHW-10	Unknown/sporadic	10 y/11 y	Moderate dHMN with UL sparing and severe foot deformity. Mild autism, ^b attention deficit, and anxiety. Normal brain MRI. AFOs (16 y). CMTPedS 27/44 (14 y).
HLF-7	Unknown/sporadic	3 y/13 y	Moderate dHMN with hand function severely affected (9 y). +++ UL + LL. Mild dysarthria (3 y). Congenital cataracts. CMTPedS 20/44 (14 y).
HLF-8	Unknown/sporadic	Birth/9 y	Moderate dHMN sparing LLs proximally and ULs. +++ LL. Foot deformity on antenatal US. Delayed walking. AFOs (<1 y). CMTPedS 15/44 (9 y).
HLF-9	Unknown/sporadic	Birth/7 y	Severe dHMN sparing LLs proximally and ULs. +++ UL + LL. Foot deformity on antenatal US. Delayed walking. Lumbar hyperlordosis. AFOs (<1 y). CMTPedS 15/44 (7 y).
HLF-10	Unknown/sporadic	Birth/14 y	Moderate dHMN. +++ UL + LL. Babinski sign. Congenital talipes equinovarus and delayed walking. CMTPedS 14/44 (14 y).
HLF-11	Unknown/sporadic	Early childhood/14 y	Mild dHMN. +++ LL. Mild fatigue (15 y). CMTPedS 11/44 (19 y).
HLF-12	Unknown/sporadic	Early childhood/13 y	Moderate dHMN with mild hand weakness (17 y). CMTPedS 23/44 (16 y).
HLF-13	Unknown/sporadic	Birth/5 y	Severe dHMN with LL arthrogryposis and neurodevelopmental delay. +++ UL + LL. Divergent squint. Severe intellectual disability. Brain MRI with delayed myelination (3 y). Bilateral hip dislocation (2 y). Severe scoliosis (9 y). AFOs + wheelchair (1 y).
HLF-14	Unknown/sporadic	2 y/4 y	Moderate dHMN sparing ULs.
HLF-15	Unknown/AD	<3 y/8 y	Moderate dHMN sparing ULs. +++ UL + LL.

Abbreviations: aa = amino acid; AD = autosomal dominant; AFO = ankle-foot-orthosis; c = change; CHW = The Children's Hospital at Westmead; CMTPedS = Charcot-Marie-Tooth disease Pediatric Scale (mild [0–14], moderate [15–29], and severe [30–44]); dHMN = distal hereditary motor neuropathy; *dn* = de novo; HLF = Hospital Universitari i Politècnic La Fe; ID = identifier; LL = Lower limb; mID = mild intellectual disability; OSA = obstructive sleep apnea; SMA-LED = spinal muscular atrophy, lower extremity dominant; UL = Upper limb; US = ultrasound; +++ = hyperreflexia.

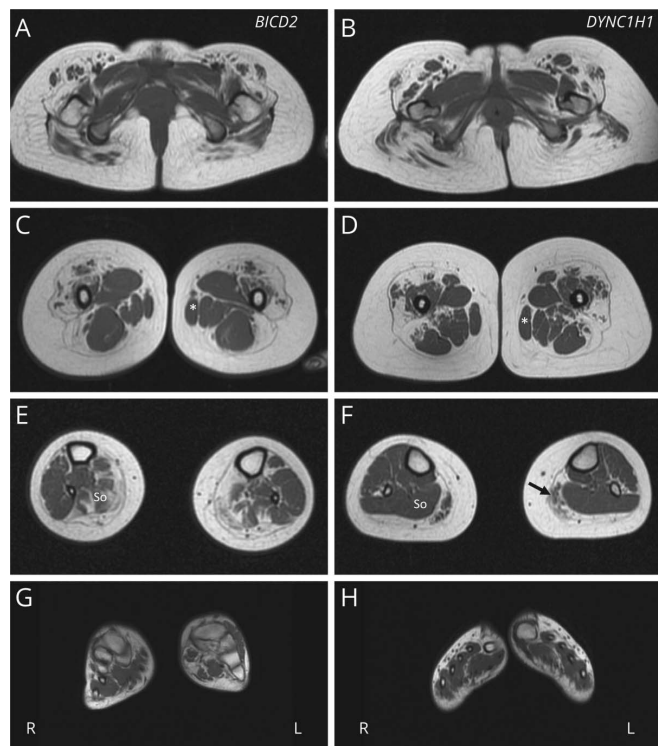
^a Novel pathogenic variant.
^b Family history of autism not associated with any neuromuscular disorder.

28.6% and 46.2% from baseline, respectively, while individuals with *BICD2* were relatively stable, showing a decrease of 8% from baseline ($n = 3$).

Discussion

dHMN represents a phenotypically and neurophysiological distinct subgroup of children with inherited neuropathy, although there is significant overlap with CMT, especially motor

CMT2. Recent studies conducted in the same region estimated that dHMN is much rarer subtype within the broader CMT group (2.14 vs 9.8 per 100,000 inhabitants).^{10,18} The lower prevalence of dHMN, the late onset of some subtypes,¹⁹ and the technical challenges of conducting sensory studies in infants²⁰ may explain the scarcity of literature on pure distal motor neuropathies in childhood. We present a large cohort of children and adolescents with dHMN studied extensively from the clinical and genetic perspective. The current study

Figure 2 Muscle MRI in Adolescents With *BICD2*- and *DYNC1H1*-SMA-LED

T1-weighted axial muscle MRIs of the entire lower limb of 2 female patients who were similar age and disease severity at the time of the MRI. (A, C, E, and G) MRI panel corresponding to patient HLF-1 (at age 15 years, Charcot-Marie-Tooth Disease Pediatric Scale [CMTPed5] total score 16, some images have been reported previously¹⁵). (B, D, F, and H) MRI panel corresponding to patient HLF-6 (at age 16 years, CMTPed5 total score 13). (A and B) Muscles of the pelvis with the most affected being the gluteus maximus, gluteus medius (not shown), tensor fascia latae, both vastus (lateralis and intermedius), rectus femoris, and sartorius. (C and D) At the proximal thigh level, the preservation of the adductors muscles (adductor longus, brevis, and magnus) and gracilis (white asterisk) is striking compared to the severe fat replacement of the anterior compartment of the thigh. (E and F) The most affected muscles of the calf were the gastrocnemius in both patients, but the soleus (So) was characteristically spared only in the *DYNC1H1*-spinal muscular atrophy, lower extremity dominant (SMA-LED). Tibialis anterior muscles were also involved in both individuals, and the peroneal muscles were not affected. Presence of small foci of normal muscle within fatty muscle tissue are indicated by a black arrow. (G and H) Intrinsic foot muscles showed mild fatty replacement in *BICD2*-SMA-LED (G) but remained preserved in the patient with *DYNC1H1* (H).

contributes to our understanding of the heterogeneous genetic basis and disability profile of childhood-onset dHMN.

In our pediatric cohort, the age at onset was variable, but about half presented in the first year of life. Most presented with foot deformity. We found *BICD2* to be the most commonly identified gene in the entire cohort, including in those who presented in the first year of life. The single individual with a pathogenic *GARS* variant had the most severe presentation and was the only genetically classified patient with an upper limb predominance. We detected mild to severe cognitive impairment in a quarter of individuals with dHMN (6 of 22).

Genetic distribution in dHMN has previously been described in only 3 predominantly adult cohorts. In 2008, 112 index patients with dHMN were analyzed and pathogenic variants in *BSCL2*, *HSPB8*, *HSPB1*, and *SETX* were found in 17.⁸ In the genetically classified group (n = 17), 8 individuals showed UMN signs; in 7, disease onset was after 17 years of age, and only 4 patients were <20 years of age when recruited.⁸ In a 20-year historic cohort of CMT2 and dHMN, only 7 of 45 presented with dHMN.⁹ In 2 of those patients, they identified pathogenic variants in *BSCL2* and *HSPB1*.⁹ In a recent cohort analysis, 64 of 105

patients had dHMN and a later onset (after 16 years of age) than our cohort.¹⁰ These adults carried pathogenic variants in a varied spectrum of genes, and *BICD2* was placed third in frequency after *IGHMBP2* and *SYT2*. Patients with pathogenic variants in small heat-shock proteins (*HSPB1* and *HSPB8*) are common in adult dHMN cohorts but almost always present with onset after second decade,²¹ with an average age at onset in the fourth decade in pathogenic *HSPB1* variants.²² Hence, childhood cohorts of dHMN differ genetically from adult cohorts, with only *BICD2* showing a high frequency in both cohorts.

The only genetically diagnosed patients with UMN signs on examination were those carrying pathogenic variants in *MFN2*. Genetic and clinical heterogeneity of *MFN2*-related neuropathies is widely known.²³ Our patient with the p.Arg259Cys change in *MFN2* differed from the 2 previously described with pathogenic variants at this codon (p.Arg259Cys and p.Arg259Leu) with an earlier age at onset, absence of optic neuropathy, and absence of sensory involvement.^{24,25} The severe dHMN of our patient with the p.Arg94Gly change in *MFN2* is in keeping with reports that pathogenic variants affecting amino acid 94 may preferentially affect motor neurons.²⁶

Table 2 Nerve Conduction Studies in Affected Children With dHMN

Patient ID	Gene aa change	Age at study, y	Median CMAP/CV	Ulnar CMAP/CV	Peroneal CMAP/CV	Tibial CMAP/CV	Median/Ulnar SNAP	Sural/sup peroneal SNAP	CD on EMG
CHW-1	<i>GARS</i> Asp205His	5	0.4 ^a /ND	2.2 ^a /36 ^a	0.1 ^a /ND	2.1 ^a /24 ^a	32/18	23/ND	ND
CHW-4	<i>BICD2</i> Ser107Leu	4	12.6/48	ND	ND	16.2/58	21/ND	45/ND	Yes ^a
HLF-1	<i>BICD2</i> Ser107Leu	15	ND	11.7/ND	7.4/55.6	14.1/ND	ND	22.6/ND	Yes ^a
HLF-2	<i>BICD2</i> Ser107Leu	9	ND	ND	5.2/52.9	12/ND	ND	23.9/ND	Yes ^a
HLF-3	<i>BICD2</i> Ser107Leu	11	ND	ND	6.2/47.3	4.5 ^a /ND	ND	19.8/ND	Yes ^a
HLF-4	<i>BICD2</i> Val485Gly	8	ND	ND	3.7 ^a /54.5	ND	ND	28/ND	Yes ^a
HLF-5	<i>BICD2</i> Tyr557His	16	8/50	8.7/62.1	4.7/45.2	6.6 ^a /46.1	14.6/10	21.3/ND	Yes ^a
CHW-5	<i>DYNC1H1</i> Arg251Cys	4	9.9/61	ND	3.4 ^a /60	9.3 ^a /55	38/ND	18/ND	No
HLF-6	<i>DYNC1H1</i> His306Arg	9	14.2/65.2	ND	12.2/57.7	24.7/ND	40/ND	40/ND	Yes ^a
CHW-6	<i>MFN2</i> Arg259Cys	9	12.1/57	9.1/58	0.9 ^a /57	1.8 ^a /42	75/97	24/ND	ND
CHW-8	<i>MFN2</i> Arg94Gly	6	9/46.5	8.3/61.5	7.3/42.1	8.5 ^a /40.5	23.5/10.7	ND	ND
CHW-10	Unknown	12	8.9/61	6.8/71	NR ^a	0.2 ^a /93	31/15	23/ND	No
HLF-7	Unknown	14	NR ^a	6.1 ^a /61.1	9.7/51.8	9.3 ^a /ND	24/11	24/ND	ND
HLF-8	Unknown	10	9/60	9.3/67.4	0.1 ^a /ND	16.6/ND	24.6/9.2	ND/26.4	ND
HLF-9	Unknown	4	ND	ND	2.8 ^a /53.4	8.3 ^a /ND	ND	ND/24	ND
HLF-10	Unknown	14	ND	15.1/58.3	6.8/45.1	15.4/ND	ND/14	23/ND	Yes ^a
HLF-11	Unknown	15	12.1/60.8	ND	9.2/50	19.1/ND	20/ND	20/19	Yes ^a
HLF-12	Unknown	11	5.6/55.1	9/55.6	NR ^a	6.9 ^a /ND	17.3/12.2	16.4/ND	Yes ^a
HLF-13	Unknown	6	3.8/63.6	4.3/62.2	NR ^a	1.4 ^a /44.3	ND/10.3	20.6/ND	ND
HLF-14	Unknown	4	ND	ND	4.4 ^a /43	9.5 ^a /44.3	ND	29/ND	Yes ^a
HLF-15	Unknown	7	14/58	ND	NR ^a	0.4 ^a /39	20/ND	16.2/ND	Yes ^a

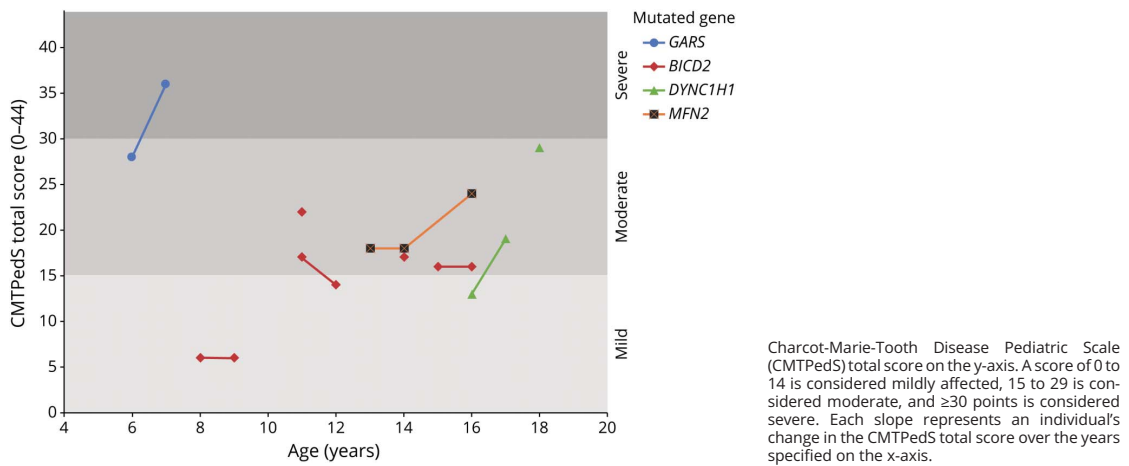
Abbreviations: Aa = amino acid; CHW = The Children's Hospital at Westmead; CMAP = compound muscle action potential (mV); CV = conduction velocity (m/s); CD = chronic denervation; dHMN = distal hereditary motor neuropathy; HLF = Hospital Universitari i Politècnic La Fe; ID = identifier; ND = not performed; NR = not recordable; SNAP = sensory nerve action potential (μ V); Sup = superficial.
^a Abnormal results >2 SD below reference values.³⁸

Most patients with dHMN caused by heterozygous pathogenic variants in the *GARS* gene present an adolescent or early-adult onset.²⁷ Infantile form has been associated with pathogenic variants only in the anticodon binding domain so far.^{28,29} We identified a novel pathogenic variant (p.Asp205His) in the catalytic domain of *GARS* in an individual with an infantile-onset dHMN.

Since the first animal models for *GARS*-induced peripheral neuropathy were described in 2015,³⁰ promising therapeutic targets such as histone deacetylase (HDAC) 6 have been

studied.³¹ HDAC6 inhibitors rescued motor phenotype in mice harboring pathogenic *GARS* variants,³² placing clinical trials as the next milestone in dHMN and indicating the need for a dHMN-specific outcome measure. While inhibition of a different group of histone deacetylases (HDAC2) showed promise in *in vivo* and *in vitro* models of spinal muscular atrophy,³³ a recent systematic analysis has shown that sodium valproate, an HDAC2 inhibitor, had only limited benefit in clinical trials, improving some motor function measures, but not others, and not improving respiratory function.³⁴ Analyses in this cohort suggest that a scale modeled on the CMTpedS might be useful in measuring functional

Figure 3 CMTPedS Total Scores for Each Patient With a Genetic Diagnosis and Progression Over Time



impairment in children and adolescents with dHMN and may be useful as an outcome measure in clinical trials.

In our cohort of 12 patients with follow-up data, the disease progressed at a rate of 1.5 ± 3.7 CMTPedS points or a 9% increase from baseline over 1 year, almost double CMT1A change over 1 year¹¹ and equivalent to 9 patients with CMT1B over 2 years.³⁵ Functional outcome measures like the CMTPedS are sensitive tools to monitor outcomes longitudinally. Families and treating doctors assessed disease course as stable, and this was true of the individuals with pathogenic *BICD2* variants.

There was wide variability in the rate of progression within the individuals in the cohort, with the individuals with the *GARS* and *DYNC1H1* showing significant worsening (28.6% and 46.2% increase from baseline, respectively) and the disease being relatively stable in those with *BICD2*-SMA-LED (8% decrease from baseline), suggesting that the rate of progression may differ between genetic subtypes of dHMN.

Balance was one of the most affected items in individuals with dHMN. This observation indicates that balance training should become a major rehabilitation trial target in dHMN and that

Figure 4 CMTPedS Item Score Frequencies at Baseline in Patients With Distal Hereditary Motor Neuropathy

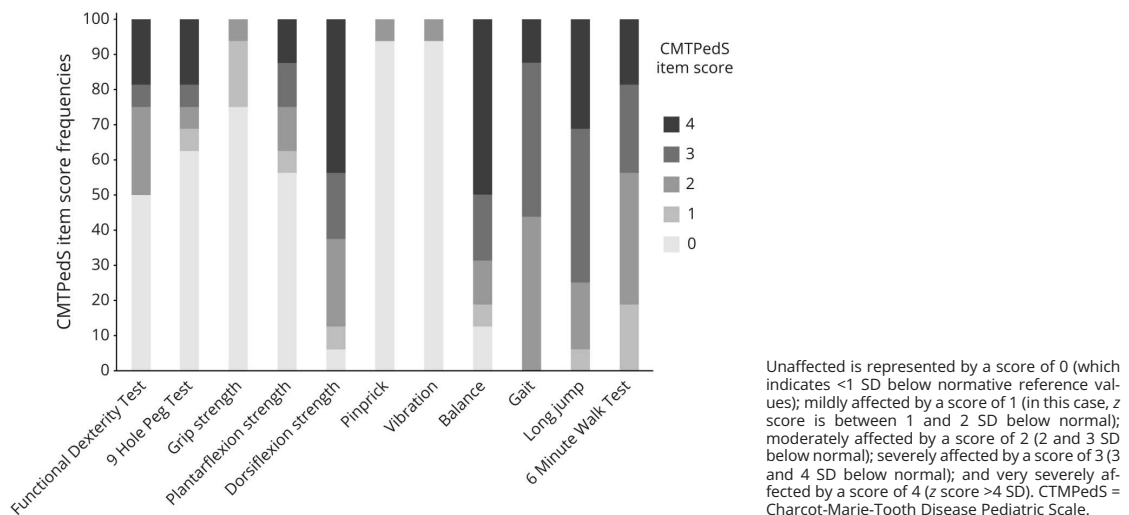


Table 3 CMTPedS Item z Score–Total Score Correlation

Item	Correlation	p Value
Functional dexterity test	0.63 ^a	0.009
9-Hole Peg Test	0.63 ^a	0.009
Grip	-0.65 ^a	0.006
Plantarflexion	-0.49	0.052
Dorsiflexion	-0.19	0.475
Balance	-0.77 ^a	0.001
Long jump	-0.86 ^a	<0.0001
6-min walk test	-0.55 ^b	0.029

Abbreviation: CMTPedS = Charcot-Marie-Tooth disease Pediatric Scale. For the functional dexterity test and 9-Hole Peg Test, because they are timed tests, a positive z score indicates a longer time and hence a worse performance. For all other items, a negative z score means a worse performance. Three items of the CMTPedS (pinprick, vibration, and gait) are converted directly to item scores and thus are not included in this analysis.

^a Correlations are significant at the 0.01 level.

^b Correlations are significant at the 0.05 level.

sensory loss might not be the main cause of poor balance in inherited neuropathies. Apart from vibration and pinprick, ankle dorsiflexion strength and foot alignment have also been shown to significantly correlate with balance in a pediatric CMT cohort.³⁶

A number of reasons support removal of sensation items from a future disability outcome measure for dHMN: these items had a floor effect in dHMN; examining pinprick and vibration in very young children is difficult; internal consistency remains unchanged when these items are excluded; and sensation items were recently omitted from a scale that measures disability in adults with CMT.³⁷ Grip strength is worth retaining because this item is affected differently in the upper limb and lower limb–dominant dHMN and deteriorates over time in the upper limb–predominant phenotype.

There are some limitations to our study. The retrospective nature of our study and the small number of patients in each genotype prevented a comparison between subtypes of dHMN. Interitem correlations and correlation of each item with the scale total score were not statistically corrected for multiple comparisons, so caution is advised in the interpretation of the results. Follow-up assessment with CMTPedS was limited to 1 year in most cases, and this may also be responsible for the lack of sensitivity to change of the CMTPedS score in specific subtypes of dHMN. Longitudinal studies with a larger cohort of patients with specific genetic subtypes of dHMN will be required to obtain an accurate measure of disease progression in these cohorts.

dHMN in childhood are rare, genetically heterogeneous, and usually slowly progressive. Pyramidal tract involvement and cognitive involvement are frequent in pediatric dHMN. The CMTPedS is a sensitive measure of disability in dHMN and shows progression over 1 year. Larger studies are required to

evaluate the rate of progression for subtypes of dHMN and to further optimize this scale as an outcome measure in childhood dHMN population for use in clinical trials.

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Disclosure

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Continued

Appendix (continued)

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A very mild phenotype of Charcot-Marie-Tooth disease type 4H caused by two novel mutations in *FGD4*



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ABSTRACT

Background: Mutations in the *FGD4* gene cause an autosomal recessive demyelinating peripheral neuropathy referred to as CMT4H, characterized by its onset in infancy or early-childhood and its slow progression.

Methods: The clinical and genetic status of two patients with CMT4H was studied, performing genetic testing with a panel of genes and analysing *FGD4* mRNA expression by quantitative PCR.

Results: Two novel *FGD4* variants (c.514delG and c.2211dupA) were identified in two mildly affected Spanish siblings with CMT4H, and with disease onset in late adolescence/adulthood (one of them remaining asymptomatic at 20). On examination, foot deformity was observed without weakness or sensory involvement, and in the muscles of the lower extremities magnetic resonance imaging showed no fat replacement. Further analysis of *FGD4* expression in peripheral blood suggested that neither mutation affected splicing, nor did they affect the dosage of *FGD4* mRNA (compared to a healthy control). It was predicted that each allele would produce a truncated protein, p.Ala172Glnfs*28 (c.514delG) and p.Ala738Serfs*5 (c.2211dupA), the latter containing all the functional domains of the native protein.

Conclusions: The conservation of functional domains in the proteins produced from the *FGD4* gene of two patients with CMT4H, could explain both the milder phenotype and the later disease onset in these patients. These results expand the clinical and mutational spectrum of *FGD4*-related peripheral neuropathies.

1. Introduction

The classification of Hereditary Motor and Sensory Neuropathies

(HMSN), commonly referred to as Charcot-Marie-Tooth (CMT) disease, as either demyelinating or axonal is usually based on electrophysiological or pathological features. Autosomal recessive

Abbreviations: HMSN, Hereditary motor and sensory neuropathies; CMT, Charcot-Marie-Tooth disease; *FGD4*, frabin gene; CMT4H, CMT type 4H; CMTNS-v2, CMT Neuropathy Score version 2; CMTpedS, CMT Paediatric Scale; MRI, Magnetic resonance imaging; dHMN, distal hereditary motor neuropathy; ALS, Amyotrophic lateral sclerosis; RT-PCR, reverse transcription-PCR; qPCR, quantitative PCR; NCV, Nerve conduction velocity; CMAP, Compound motor action potential; SNAP, Sensory nerve action potential; DL, Distal latency; NR, Non recordable; ns, No significant; FAB, F-actin binding; DH, Dbl homology; PH, Pleckstrin homology; FYVE, Fab 1, YOTB, V ac 1, and EEA1 zinc finger domain; NMD, Nonsense-mediated decay

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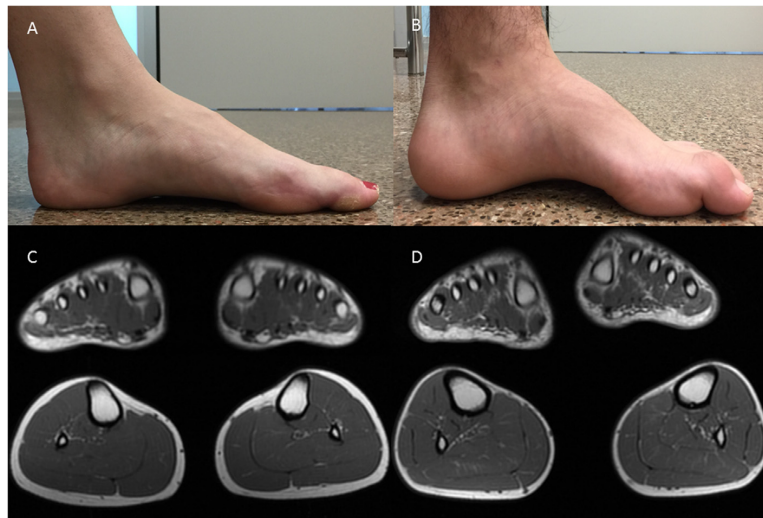


Fig. 1. Clinical images and muscle MRI (T1 weighted images shown). A Mild pes cavus of patient II:1. B Moderate pes cavus with hammer toes of patient II:2. C Muscle MRI of patient II:1 showing no fat replacement at the level of the feet and calf. D Muscle MRI of patient II:2 showing no significant abnormalities of either the feet or calf.

demyelinating forms of CMT (CMT4) tend to produce a more severe phenotype than autosomal dominant demyelinating forms (CMT1), and with an earlier onset [1,2]. In 2007, biallelic mutations in the frabin gene (*FGD4*) that encodes a Rho GTPase guanine nucleotide exchange factor were identified as the cause of CMT type 4H (CMT4H) [3]. Since then, 31 patients from 19 families with CMT4H have been reported, the vast majority of whom experienced symptoms during childhood (usually before the age of 3) or presented with delayed walking [4]. In some patients, scoliosis [3,10,12,13,15,16,18], and sensory ataxia [4,11,13] have been observed. Individual CMT4H cases have recently been reported with spinal syringomyelia [11], pupil asymmetry [8], multiple cranial nerve involvement [14], or cerebellar dysfunction [18]. Here we describe two patients with CMT4H, one of whom was asymptomatic at the age of 20, that carried two novel, compound heterozygous frameshift mutations in *FGD4*. An analysis of these mutations sheds light on their possible implications for the phenotype of these patients.

2. Material and methods

2.1. Subjects

The two affected individuals are siblings born to a healthy non-consanguineous Spanish couple (Fig. 2A) with no family history of neuromuscular disease. A thorough neurological examination was carried out at the Neuromuscular Unit of the Department of Neurology of the Hospital Universitari i Politècnic La Fe (HUPLF), where the two individuals were subsequently followed. The phenotype of each individual was studied using two scales designed to measure disability in inherited sensory-motor neuropathies: the CMT Neuropathy Score version 2 (CMTNS-v2) and the CMT Paediatric Scale (CMTPedS). CMTNS-v2 includes neurophysiology items and it has been validated for patients older than 16. The scores obtained range from 0 to 40 and patients are classified as mild with a CMTNS-v2 score ≤ 10 [5]. CMTPedS is an 11-item scale for patients between 3 and 20 years of age in which the scores range from 0 to 44, with a score of 0 representing unaffected patients [6]. Flexibility of ankle joint dorsiflexion was measured weight bearing using the lunge test. No Achilles retraction is present if lunge test $> 35^\circ$ [7]. Both patients also underwent comprehensive electrophysiological studies and muscle magnetic resonance imaging (MRI) was performed on the hips, thighs, calves and feet using a 3-T system (Siemens Vision, Siemens, Germany). After obtaining

informed consent, blood samples were collected from all five members of the family and DNA was extracted using standard procedures. This study was approved by the institutional research board (IRB) at the Health Research Institute Hospital La Fe.

2.2. Molecular studies

Patient II:2 was tested for our customized panel of 119 genes using SureSelectQXT technology for Illumina (Agilent Technologies, Santa Clara, CA, USA), a panel that includes genes associated with CMT, distal hereditary motor neuropathy (dHMN), and familial amyotrophic lateral sclerosis (fALS). Cascade testing (using Sanger sequencing) was performed for other family members. To study the possible effect of these two variants on mRNA expression, we analysed the cDNA products generated from the *FGD4* mRNA extracted from the peripheral blood of patient II:2 and his progenitors. After extracting total RNA using the PAXgene Blood RNA kit (QIAGEN, Valencia, CA, USA), cDNA was obtained and amplified by reverse transcription-PCR (RT-PCR) using the qScript cDNA SuperMix (Quantabio, Beverly, MA, USA). The presence of mutations at the cDNA level was determined by Sanger sequencing with the following forward and reverse primers: 5' CAGATCTCATCAGTCGCTTTG and 5' TGCTTCTTCCACAGTTTGC to study the c.514delG variant; and 5' CATAAGTGGATTTCACAGACAGTG and 5' GAATGACTCTGCACACTAATTTTC to study the c.2211dupA variant. To analyse the relative amounts of the cDNA products, quantitative PCR (qPCR) was performed using the Perfecta SyberGreen Mix (Quantabio, Beverly, MA, USA) and the following forward and reverse *FGD4* primers: 5' TCAGATCTCATCAGTCGCTTTG and 5' ACAGCAGACTCTTCTTCAAA TCA. A healthy control sample was used for calibration and *GAPDH* was used as the reference gene for normalization. An unpaired *t*-test was used to compare the cDNA doses in II:2 with those in the rest of the samples.

3. Results

3.1. Clinical picture

The older of the two patients (Fig. 2A, II:1) studied here is a 20-year-old woman who remains completely asymptomatic, displaying no difficulties in running, jumping or handling small objects. Since childhood, she had trained for 10 h each week as a rhythmic gymnast, with no limitations. At the age of 16 she began to perform highly demanding

Table 1
Motor and sensory nerve conduction studies.

CMAP				Patient II:1 (20 yo, F)			Patient II:2 (17 yo, M)		
Nerve	DL (ms)	Amplitude (mV)	NCV (m/s)	DL (ms)	Amplitude (mV)	NCV (m/s)			
Median	5.5 [4.5]	11.6 [5.9]	21.1 [53]	6.45 [4.5]	4.3 [5.9]	16.5 [49]			
Ulnar	4.6 [3.7]	6.9 [7.9]	22.2 [52]	5.0 [3.7]	5.3 [7.9]	14.9 [52]			
Peroneal	9.2 [6.5]	3.1 [2.6]	15.0 [43]	12.2 [6.5]	3.8 [2.6]	12.0 [43]			

SNAP			Patient II:1 (20 yo F)		Patient II:2 (17 yo M)	
Nerve	Amplitude (µV)	NCV (m/s)	Amplitude (µV)	NCV (m/s)		
Median	15.9 [17]	27.8	8.2 [17]	22.7		
Ulnar	6.4 [14]	27.9	2.4 [14]	22.3		
Radial	18 [7]	27.8	5.2 [7]	22.4		
Sural	3.2 [4]	24.0	NR [4]	NR		

CMAP, Compound motor action potential; DL, Distal latency; ms, millisecond; mV, millivolts; NCV, Nerve conduction velocity; m/s, metres per second; SNAP, Sensory nerve action potential; µV, microvolts; NR Non recordable. Lower limits of onset-to-peak amplitudes and velocities are shown as mean – 2 SD in box brackets. Reference values were extracted from Chen S, Andary M, Buschbacher R, Del Toro D, Smith B, So Y, et al. Electrodiagnostic reference values for upper and lower limb nerve conduction studies in adult populations. *Muscle Nerve* 2016;54:371-7.

cardiovascular exercise three times weekly. She does not need to use any special apparatus or require special footwear, although areflexia and mild pes cavus were detected at 19 years of age (Fig. 1A), as well as mild retraction of the Achilles tendons (lunge test 30° on the left side and 25° on the right) that mildly affected heel walking. The patient's motor balance, sensory examination and muscle mass were normal, and neither pupillary abnormalities nor scoliosis were detected. The CMTNS-v2 score for this patient was 0, while the total CMTPedS score was 1 (scoring 1 on gait). Electrophysiological studies showed slow motor and sensory nerve conduction velocities (NCVs) in all nerves. Amplitude of compound muscle action potentials (CMAPs) and sensory nerve action potentials (SNAPs) was normal or marginally reduced, respectively (Table 1). The muscle MRI performed at age 19 revealed no fat replacement or volume changes, not even in her feet (Fig. 1C).

The index patient (Fig. 2A, II:2) was a 17-year-old male who walked unassisted at 12 months of age, yet he began to suffer from dorsal kyphoscoliosis and increased plantar arch when he was 11 years old. He was prescribed shoe-inserts when aged 12 and indicated he was symptomatically stable since the age of 13. From the age of 3 until he was 16, he had been playing football for up to 12 h per week, having suffered no injuries or experiencing difficulties in keeping up with his peers. When examined at the age of 16, areflexia, moderate pes cavus and hammer toes were noted (Fig. 1B), and a lunge test was compatible with mild Achilles tendon retraction (20° on both sides). No weakness, amyotrophy, pupillary size abnormality or sensory deficits were observed. He scored 4 in the CMTNS-v2, mainly because of the neurophysiological alterations, and his total CMTPedS Score was 3, scoring 2 on balance and 1 on gait. His nerve conduction was similar to that of his sister, although his motor NCVs were slower, and the amplitude of his CMAPs and SNAPs was smaller (Table 1). Muscle MRI of lower extremities at the age of 16 did not show any significant abnormalities (Fig. 1D).

3.2. Genetic analysis

Genetic testing of the proband (Fig. 2A: II:2) revealed two novel candidate variants in the *FGD4* gene (NM_139241.2): c.514delG and c.2211dupA. The other three variants were identified in heterozygous status: one missense variant in *PLEKHG5* (rs140202670), and a synonymous change in both *SBF1* and *UBQLN2* (rs180800708 and rs142250604, respectively). Their allele frequency were relatively high in the control database consulted (ExAC and gnomAD). Sequence variants in *SBF1* and *UBQLN2* were classified as benign based on the American College of Medical Genetics and Genomics criteria. The

variant in *PLEKHG5* gene was ruled out as disease-causing because known mode of inheritance is autosomal recessive. In contrast, both *FGD4* mutations were absent in the control and mutation databases (ExAC, gnomAD, NCBI, ClinVAR and HGMD) and segregated with the disease within the family. Segregation analysis confirmed that these two changes exist in *trans* and while the unaffected mother harboured the c.514delG variant in heterozygosis, the healthy father carried the c.2211dupA variant in heterozygosis. Patient II:1 also carried both mutations, whereas the healthy sibling (Fig. 2A: II:3) did not carry either. The presence of both changes in heterozygosis was confirmed by analysing the *FGD4* mRNA isolated from patient II:2, sequencing two different cDNA fragments and thereby ruling out any alterations in the mRNA sequence adjacent to both mutations (Fig. 2B). In addition, qPCR analysis of *FGD4* mRNA in patient II:1 showed that there were no significant differences in the *FGD4* mRNA dosage in the tissue examined relative to the healthy control, the unaffected carriers or patient II:2 (Fig. 2C). It was predicted that the two variants identified each produce a truncated protein, the p.Ala172Glnfs*28 (c.514delG) that lacks functional domains and the p.Ala738Serfs*5 (c.2211dupA) that contains all of these (Fig. 3).

4. Discussion

We have identified two novel frameshift mutations in the *FGD4* gene of two siblings, both diagnosed with a demyelinating neuropathy with later onset and a milder phenotype than those reported previously. Analysis of mRNA expression did not show any effect on splicing or on *FGD4* dosage, which might reflect nonsense-mediated mRNA decay (NMD).

Since the first two families with CMT4H were described [3], this condition has been regarded as a very early onset demyelinating disease with a severe phenotype that involves delayed walking, scoliosis, and severe muscle weakness associated with an early loss of ambulation. However, as the number of CMT4H families has increased (now reaching 19 in total), milder forms of this condition have also been described. In addition, a few patients with associated sensory ataxia [4,11,13], spinal syringomyelia [11], pupil abnormality [8], cranial nerve involvement [14], or cerebellar dysfunction [18] have been reported, hence broadening the known CMT4H phenotype. A clinical feature common to all patients is onset during infancy and slow disease progression. As such, all patients began to experience symptoms before they reached 9 years of age [8–18]. However, our 20-year-old patient was still asymptomatic although the symptoms began to appear in her brother during his second decade. Muscle MRI findings support the

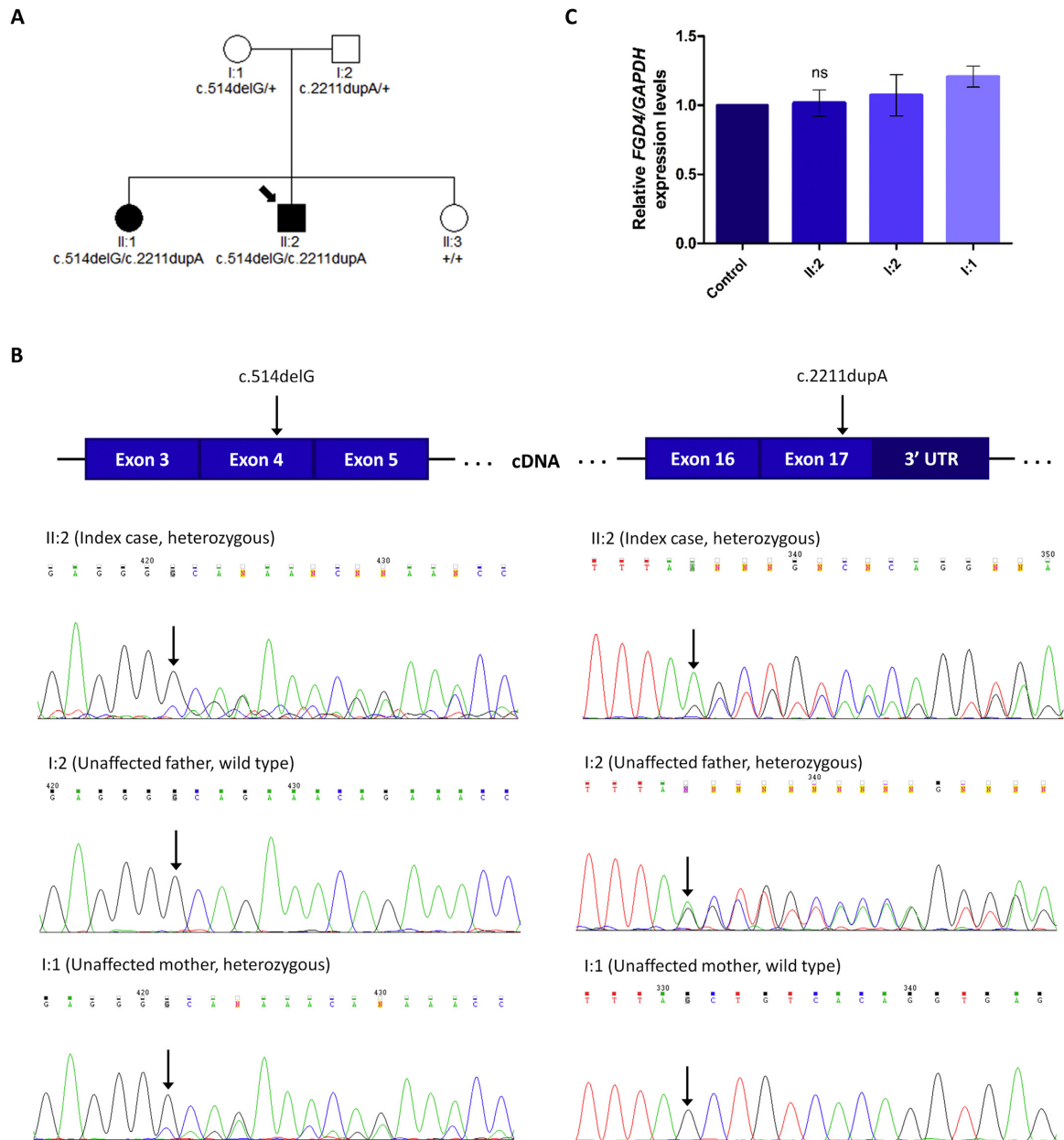
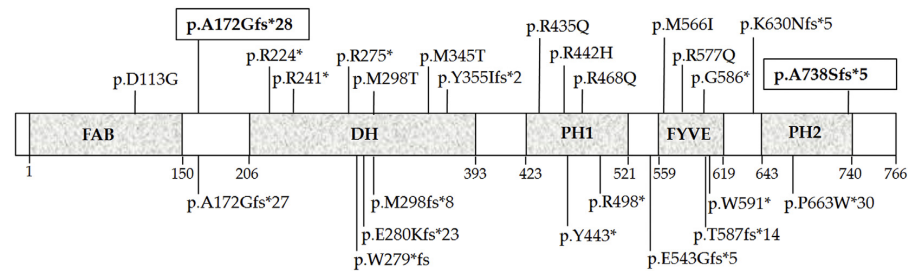


Fig. 2. Expression of the *FGD4* mutations in peripheral blood cells from the HUPLF965 family. A Family pedigree and *FGD4* genotypes. B Sequencing of the RT-PCR products obtained. Both mutations were evident in the cDNAs, as indicated by the arrow. C The qPCR analysis of *FGD4* mRNA from peripheral blood of the progenitors (I:1; I:2), index case (II:2) and the healthy control used as a calibrator. *GAPDH* was used as the reference gene for normalization and no significant (ns) differences were observed between index case II:2 and the rest of the samples (unpaired *t*-test, *p* > .05).

clinical phenotype of our patients, since they showed no signs of muscle atrophy or fat replacement. By contrast, muscle imaging in other patients with a mild phenotype revealed fat replacement in the anterior tibialis muscle [12], as well as atrophy of the anterior tibialis and hamstring muscles in a 10-year-old [9]. In our patients, NCVs were slow, a characteristic of CMT4H. However, their SNAPs were fairly well preserved, in contrast to the absence of SNAPs in the 31 nerve

conduction studies reported previously, 26 of which were performed before or during adolescence [3,9–11,15–18].

Our genetic data indicate that neither mutation affects splicing or provokes the degradation of *FGD4* mRNA in the peripheral blood sample. Hence, each allele may produce a different truncated protein, although our analysis could not rule out whether or not these proteins may be degraded prematurely. Moreover, mRNA processing could be



Variants described with unknown effect on the protein: [c.1512-2A>C;p.?]; [c.1192-48_1233del;p.?]

Fig. 3. Distribution of the pathological mutations reported in the *FGD4* protein. The mutations identified in our patients are indicated in bold and in boxes. Five functional domains are represented for *FGD4*, the FAB (F-actin binding), DH (Dbl homology), PH (Pleckstrin homology) and FYVE (Fab 1, YOTB, V ac 1, and EEA1 zinc finger) domains.

tissue-specific. Consequently, how these mutations affect splicing or degradation of mRNA in the peripheral nerve would still remain unknown. In lack of nerve tissue, investigation of mRNA processing and/or endogenous protein levels in other biological samples (i.e. from oral swab or skin biopsy) could be helpful. Considering the two truncated proteins predicted, the loss of functional domains in the p.Ala172Glnfs*28 protein means it is likely to be only weakly active at best, whereas the truncated p.Ala738Serfs*5 protein may partially have conserved *FGD4* activity since the main functional domains are retained. To date, the majority of *FGD4* variants are loss-of-function alleles that have been identified in homozygosity, and the mutations identified previously were at positions that differed from those in our patients p.Ala738Serfs*5 allele, lying more 5' in the primary sequence. It is worth considering the p.Lys630Asnfs*5 variant in more detail, a variant found in homozygosity in three patients from two unrelated families: two siblings of Spanish origin [11] and a Turkish patient [18]. The Spanish siblings were said to have experienced symptoms from when they initiated independent walking [11], whereas the Turkish patient reported his first symptoms in the second decade of life. However, this latter individual experienced proximal weakness and cerebellar dysfunction when he was 28 years old, although clinical details about his phenotype are scarce [18]. The protein produced from the p.Lys630Asnfs*5 allele would lack part of the PH2 domain, whereas our patients' p.Ala738Serfs*5 allele would generate a truncated protein that maintains this domain. Indeed, the c.2211dupA variant produces a larger protein (p.Ala738Serfs*5), the largest truncated protein as yet described in CMT4H patients, which may be partially functional and hence explain the later onset and milder phenotype in our patients. Nevertheless, we cannot ignore the influence of environmental factors on gene expression (e.g., physical activity) and how the regular intense exercise undertaken by our patients could affect their clinical presentation.

5. Conclusions

The patients presented here carrying the c.514delG (p.Ala172Glnfs*28) and c.2211dupA (Ala738Serfs*5) mutations in *FGD4* had a very mild phenotype, as witnessed by electrophysiological and MRI examination. The in depth phenotyping and comprehensive genetic analysis carried out helps us to understand the pathogenic mechanisms associated with the different mutations and their influence on the final phenotype.

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Availability of data and materials

Please contact authors for data requests.

Authors' contributions

Conceived and designed the study: HAE, VL, TS. Clinical description and supervision of patients: HAE, MF, EMS, IP, MT. Performed the experiments and analysed the results: ASM, DMR, VL. Interpretation of the results: HAE, VL, CE, TS. Wrote the manuscript: HAE, VL, TS, with input from all other authors. All authors read and approved the final version of the final manuscript.

Ethics approval and consent to participate

This study was approved by Institutional Research Board of the Institute of Health Research, Hospital La Fe. All the protocols followed in this study complied with the ethics guidelines of the journal and the institutions involved. All patients and family members reported here gave their informed consent prior to commencing the study.

Consent for publication

Consent for publication was obtained from the patients and family members.

Competing interests









The authors have no conflict of interests to declare.

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D. ARGENTE-ESCRIG H, VILCHEZ JJ, FRASQUET M, ET AL. A NOVEL TRMT5 MUTATION CAUSES A COMPLEX INHERITED NEUROPATHY SYNDROME: THE ROLE OF NERVE PATHOLOGY IN DEFINING A DEMYELINATING NEUROPATHY. NEUROPATHOL APPL NEUROBIOL. 2022;E12817.

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 neurophysiological 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

There is no statistical analysis in this work.

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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.¹ Peripheral neuropathy can either be the dominant feature of a condition (primary neuropathy) or be part of a more complex syndrome.² 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.³ 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.⁴ 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.⁵ The mtDNA encodes for 22 transfer RNAs (mt-tRNAs) that can each undergo post-transcriptional modifications.⁶ 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.⁷ 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.^{8,9} Here, we describe the detailed phenotype of three apparently unrelated patients who carry compound heterozygous mutations in

Key points

- We describe a new *TRMT5* phenotype consisting on delayed motor development, short stature, intellectual disability, mild cerebellar ataxia and severe demyelinating neuropathy.
- A wide variety of myelin abnormalities including hypomyelinated fibres, uncompact myelin lamellae and focal myelin folding can be found on nerve electron microscopy.
- This study expands the phenotype of mitochondrial disorders caused by *TRMT5* mutations and defines a new form of recessive demyelinating neuropathy.

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,

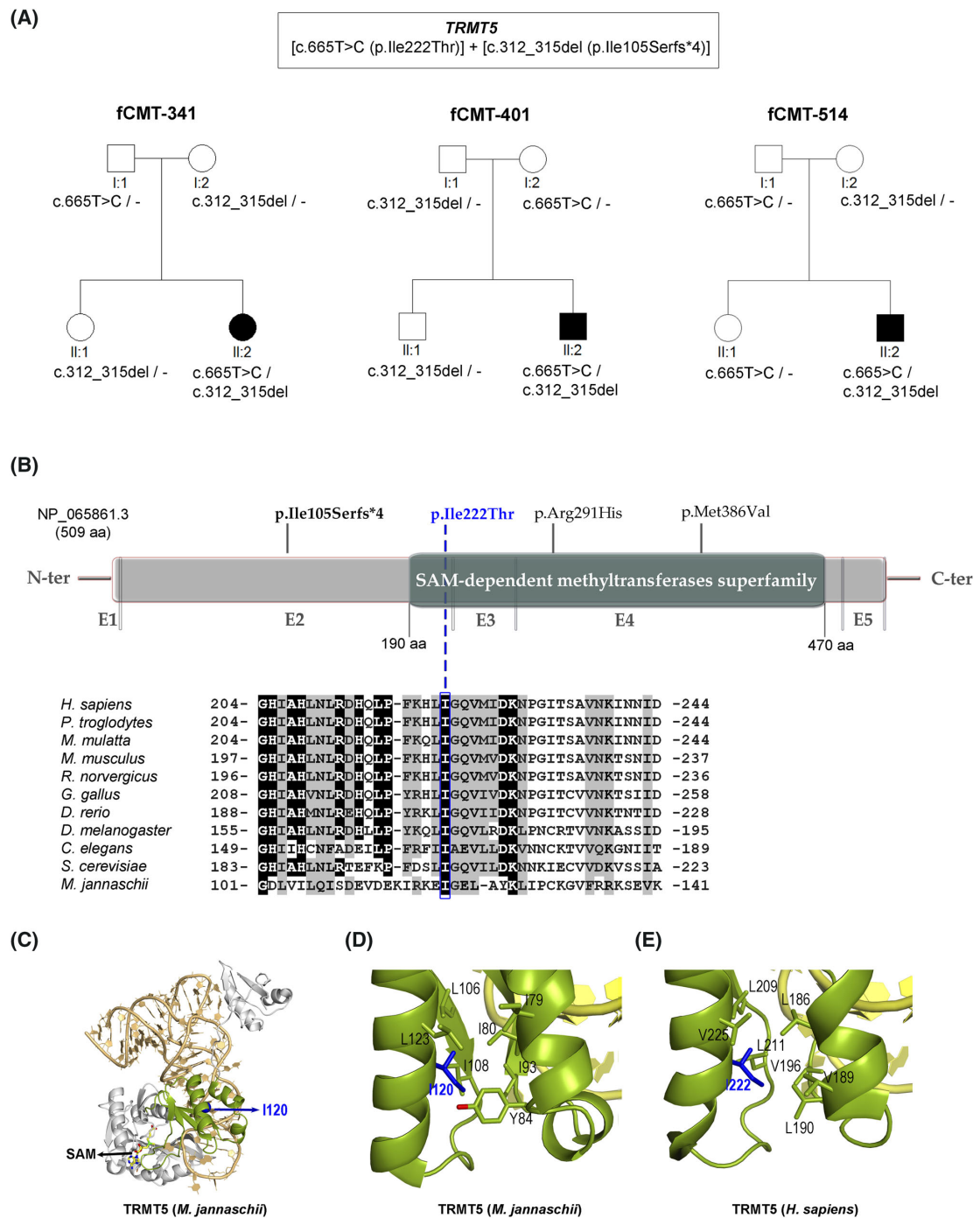


FIGURE 1 Legend on next page.

FIGURE 1 Summary of the genetic findings. (A) Pedigrees of the three unrelated families harbouring the two compound heterozygous variants in TRMT5. (B) Scheme of the TRMT5 protein domains obtained with the ScanProsite tool (<https://prosite.expasy.org/scanprosite/>) and detail of the multiple alignment of the TRMT5 protein (NP_065861.3) showing the strong conservation of the mutated Ile222 residue across different species. (C) Ribbon representation of the crystal structure of TRMT5 from *Methanocaldococcus jannaschii* complexed with tRNACys and S-adenosylmethionine: tRNACys is in orange; and S-adenosylmethionine is presented as a stick model with the carbon, nitrogen, oxygen and sulfur atoms in yellow, blue, red and green, respectively. The D1 and D3 domains of TRMT5 are in grey whereas the D2 domain is in green. A blue sphere marks the location of I120, the equivalent residue to human I222. (C–E) Detailed views of this structure (D) and of the structural model of human TRMT5 (E). The side chains of Ile 120 (D) or Ile222 (E) are shown as sticks in blue. The side chains of some surrounding residues in each of the structures are also shown and labelled

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 PCR amplification of the whole mtDNA molecule using the primers pair 5'-CCGCACAAGAGTGCTACTCTCCTC-3' and 5'-GATATTGATTTACCGAGGATGGTG-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 > C; m.10191 T > C; m.13513G > A; m.13514A > G; m.11777C > 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

TABLE 1 Clinical characteristics of the individuals carrying TRMT5 mutations

Patient age at onset (sex)	Onset	Early features	Functional status (age)	Pattern of muscle weakness on last exam						UMN features	Cerebellar features	Intellectual disability	Additional features
				UL prox	UL distal	LL prox	LL distal	UL prox	UL distal				
F1/II-2 17 (F)	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 palmonental 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 15 (M)	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 9 (M)	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.

index patients, and sensorimotor neuropathy was a prominent feature of all the individuals affected.

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 palmomental 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 vermian and hemispheric cerebellar atrophy, which was more prominent in the

TABLE 2 Nerve conduction studies in children with peripheral neuropathy associated with TRMT5 mutations

Patient ID	Age	Motor nerve conduction						Sensory nerve conduction														
		Peroneal nerve EDB			Median nerve			Ulnar nerve			Axillary nerve			Sural nerve			Radial nerve			Median nerve		
		CMAP	CV	DML	CMAP	CV	DML	CMAP	CV	DML	CMAP	DML	SNAP	SNAP	SNAP	SNAP	SNAP	SNAP	SNAP	SNAP	SNAP	
F1/II:2	7 years	2.1 (2.3)	22.2 (46)	4.1 (4.7)	8.2 (2.1)	31.5 (48)	3.6 (5.2)	8.3 (5.7)	35.1 (53)	3.6 (2.7)	-	NR	-	NR	-	NR	-	NR	-	NR	-	NR
	12 years	1.2 (2.6)	20.2 (45)	5.9 (5.6)	7.7 (5.7)	31.9 (51)	5.6 (4.9)	8.7 (7.0)	30.4 (54)	5.0 (3.1)	-	NR	-	NR	-	NR	-	NR	-	NR	-	NR
	17 years	0.6 (3.2)	20.2 (44)	4.3 (5.8)	7.5 (7.3)	32.1 (54)	4.6 (4.0)	6.3 (8.0)	32.6 (54)	3.5 (3.1)	10.6	NR	-	NR	-	NR	-	NR	-	NR	-	NR
F2/II:2	19 months	2.3 (1.7)	27 (41)	2.3 (2.9)	5.3 (2.4)	30.0 (38)	2.7 (2.7)	-	-	-	-	NR	-	NR	-	NR	-	NR	-	NR	-	NR
	11 years	NR	NR	NR	9.1 (5.7)	27.9 (51)	3.6 (4.9)	7.7 (7.0)	29.8 (54)	4.0 (3.1)	-	NR	-	NR	-	NR	-	NR	-	NR	-	NR
	15 years	NR	NR	NR	8.0 (7.3)	25.4 (54)	4.7 (4.0)	5.3 (8.0)	29.4 (54)	4.0 (3.1)	9.5	NR	-	NR	-	NR	-	NR	-	NR	-	NR
F3/II:2	3 years	2.5 (2.1)	22.5 (43)	4.2 (4.7)	5.2 (5.2)	29.6 (40)	3.7 (3.0)	6.3 (3.8)	33.8 (44)	2.9 (2.8)	-	NR	-	NR	-	NR	-	NR	-	NR	-	NR
	8 years	-	-	-	3.2 (2.1)	20.1 (48)	6.0 (5.2)	7.2 (5.7)	23.7 (53)	4.6 (2.7)	11.1	NR	-	NR	-	NR	-	NR	-	NR	-	NR

Note: Bold letters signify abnormal values, <5th percentile for amplitude and conduction velocity or >95th percentile for distal motor latency, normal values are within parentheses (Ryan CS, Conlee EM, Sharma R, Sorenson EJ, Boon AJ, and Laughlin RS: Nerve conduction normal values for electrodiagnosis in paediatric patients. *Muscle Nerve*, 2019;60:155-160). Abbreviations: n, nerve; EDB, Extensor digitorum brevis; CMAP, compound motor action potential (mV); CV, conduction velocity (m/s); DML, distal motor latency (ms); SNAP, sensory nerve action potential (µV); NR, not recordable; -, not done.

older children (Figure 2C, E). Small hyperintense foci in the subcortical white matter could also be observed at the supratentorial level in the older individuals (Figure 2B, D). Muscle MRI at the foot level showed global atrophy of the intrinsic muscles that were apparently only mildly infiltrated by fat (Figure 3L). All cases manifested prominent fat replacement in the peroneus longus muscle of the legs, along with a variable degree of fat replacement in the outer posterior compartment (Figure 3I). An overall loss of volume was evident at the level of the thigh (Figure 3F).

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.⁸ 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 tRNACys 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 (Figure 1), stabilising the folding of the D2 domain that would be expected to be altered by substituting Ile222 with a polar residue like Thr.

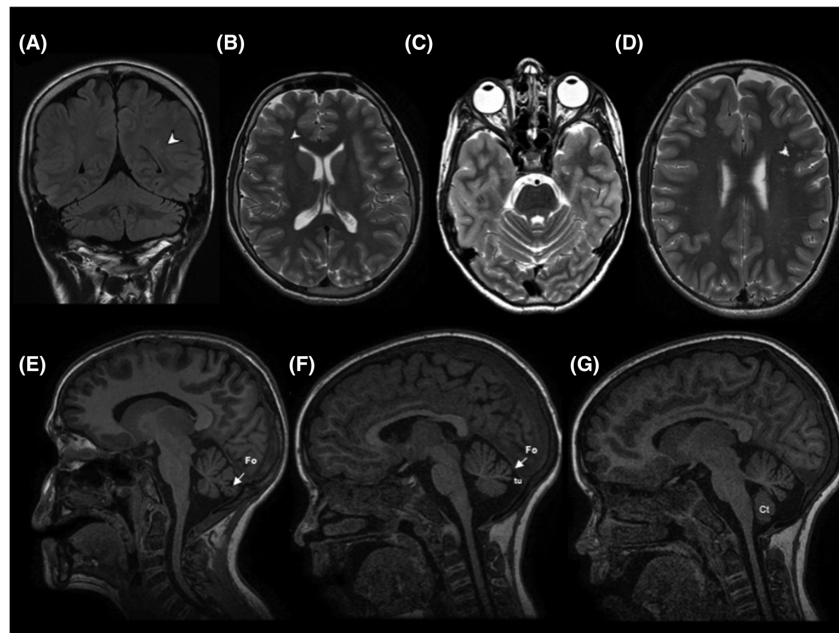


FIGURE 2 Brain MRI findings in the three children with mutations in the *TRMT5* gene. (A, B) MRI study of patient F1/II:2 (aged 17 years) showing cerebellar hemispheric atrophy (A) and scattered foci in the subcortical white matter (white arrowheads) of the parietal (A) and frontal (B) regions. (C, D) 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 (Ct)

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 Gömöri trichome 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).

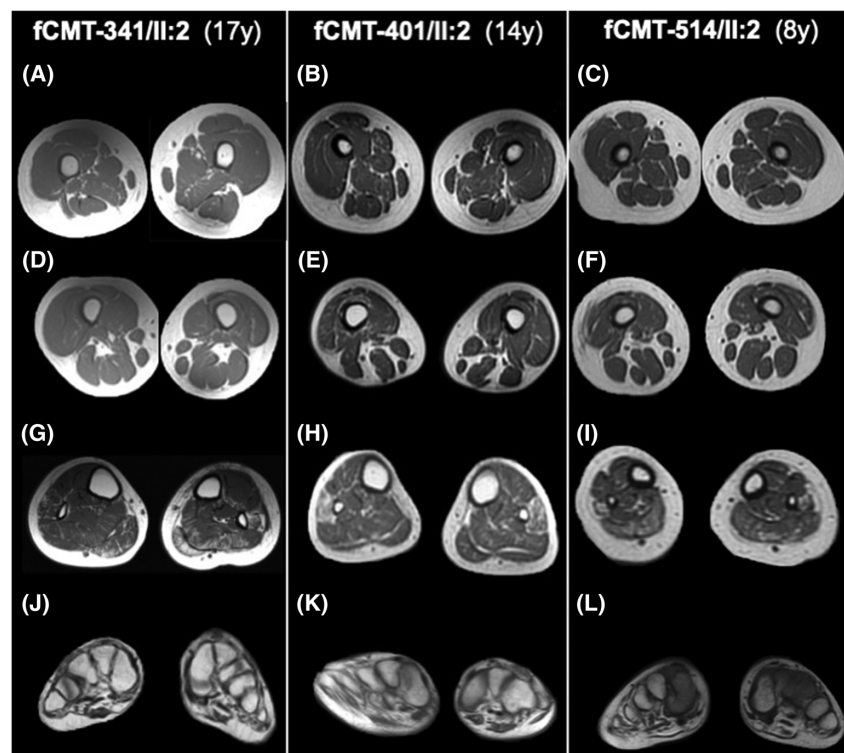
Nerve biopsy

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 uncompact lamellae (Figure 4), thus giving the impression of a delayed or arrested myelination 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 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,^{8,9} featuring exercise intolerance, lactic acidosis and evidence of multiple MRC deficiencies in skeletal muscle. Variable clinical findings included cardiomyopathy and a failure to thrive.⁸ Some of these patients developed neuropathies after decades of evolution,^{8,9,14} 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,¹⁵ 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*.^{16,17} Patients with *SURF1* defects generally display gait ataxia, growth failure, developmental regression, lactic acidemia and sensorimotor neuropathy, either axonal or demyelinating (Leigh syndrome).¹⁸ However, two families with severe childhood-onset neuropathy with a MNCV <25 m/s and lactic acidemia have been described.¹⁹ 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.²⁰ Recessive mutations in *PTRH2* have been linked to an infantile multisystem disorder that included demyelinating sensorimotor neuropathy, sensory neuronal hearing loss, cerebellar hypoplasia and exocrine pancreatic failure.²¹ However, mutations in these genes produce many systemic alterations and neuropathy is not one of the main characteristics of the phenotype.¹⁶⁻²¹ 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

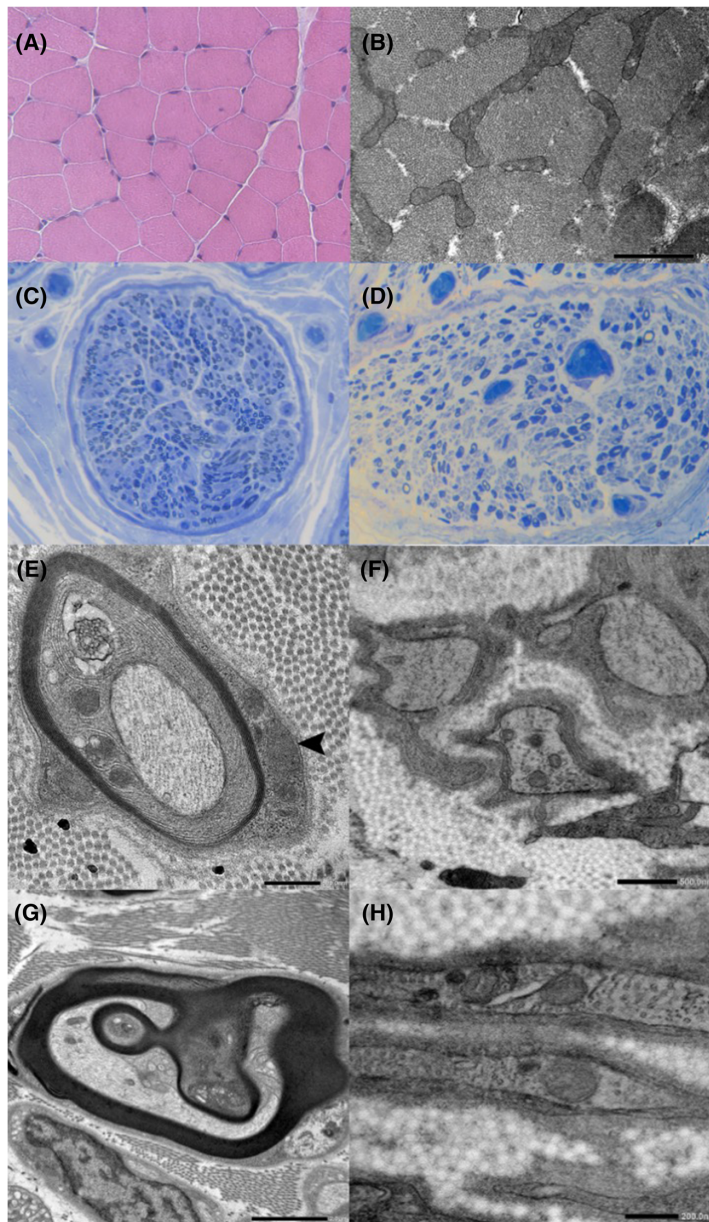


FIGURE 4 Muscle and sural nerve biopsy findings. (A, B) The deltoid muscle from patient F2/II:2 showed no abnormalities on haematoxylin-eosin staining (A), although large fused mitochondria were evident occupying much of the intermyofibrillar space on ultrastructural imaging (B). Left column (C, E, G) corresponds to the sural nerve from patient F2/II:2 at 8 years of age and the right column (D, F, H) represents the sural nerve of individual F3/II:2 at 9 years of age. (C) Semithin section showing a moderate loss of fibres, most of them small. (D) Semithin section showing a moderate loss of fibres that remain sparsely myelinated. (E) Electron microscopy 6000 \times , image in which aberrant myelination is evident, with fragments of myelin arrested within the Schwann cell that did not fold properly, an arrowhead points to a giant mitochondrion in the Schwann cell cytoplasm. (F) Electron micrograph displaying a group of three small nerve fibres whose axons are covered by a very thin myelin sheath, probably representing a delay or arrest in myelination; note as well the profuse cytoplasm of the embedding Schwann cells and their bizarre extensions. (G) Electron micrograph 4000 \times showing remarkable abnormalities of the myelin sheaths, either in their compaction (split of myelin folds) or shaping (infolding and outfolding). (H) Longitudinal section in which two incipient myelinated axons display large mitochondria in the axoplasm. Scale-bars: 500 nm in (E) and (F), 2 μ m in (G), and 200 nm in (H)

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.²² 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.²³

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

TABLE 3 Comparison of all known patients carrying biallelic TRMT5 mutations

Reference	Patient ID-sex	TRMT5 variants	Histochemical		Age at onset	Clinical picture	NCS results	Neuroimaging findings
			RCC deficiency	COX defect/lactic acidosis				
Powell CA, Kopajtich R, DSouza A, et al. TRMT5 mutations cause a defect in post-transcriptional modification of mitochondrial tRNA associated with multiple respiratory-chain deficiencies. <i>Am J Hum Genetics</i> . 2015;97:319–328	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 Systemic involvement (after 3rd decade): exocrine pancreatic failure, renal tubulopathy, cirrhosis. Cognitively normal.	Needle EMG and motor NCV normal (aged 27 years).	NA
Tarnopolsky M, Brady L, Tetreault M, Consortium F. TRMT5 mutations are associated with features of complex hereditary spastic paraparesis. <i>Neurology</i> . 2017;89:2210–2211	65,205-M	c.[312_315del;1156A > G], p.[Ile105Serfs*4; Met386Val]	I and IV	None	Birth	Global developmental 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.
	1-F	c.[312_315del; 872G > A], p.[Ile105Serfs*4; Arg291His]	I + III and IV	Marked COX deficiency (50%)/Yes	Childhood	Exercise intolerance, spasticity, and neuropathy (after 5th decade).	NCS (27 years): normal. NCS (43 years): sensory predominant axonal neuropathy	NA
	2-F		NA	No COX-negative fibres/Yes	Childhood	Global developmental delay. Required educational assistance at school. Diagnosed as cerebral palsy. MELAS-like presentation at 42. Dementia. At 33 years of age, severe distal lower limb weakness and spasticity. Systemic involvement: optic atrophy, uteropelvic junction stenosis (aged 42), no cardiac involvement.	NA	Brain MRI: a stroke crossing vascular territories.
Present work	F1/II:2-F F2/II:2-M F3/II:2-M	c.[312_315delAAATA; 749 T > C].	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)

TABLE 3 (Continued)

Reference	Patient ID-sex	TRMT5 variants	RCC deficiency	Histochemical COX defect/lactic acidosis	Age at onset	Clinical picture	NCS results	Neuroimaging findings
		p.Ile105Serfs*4; Ile222Thr		mildly elevated in F2/Ii:2)		Moderate to severe intellectual disability.		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.

neuropathies particularly those associated with congenital hypomyelinating phenotypes, such as PMP, MZP and others,²⁴ 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,⁸ 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,²⁵ and 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,^{25,26} 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.

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 an OXPHOS 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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eTable 1 Mitochondrial respiratory chain enzyme activities in skeletal muscle homogenates from the three probands

Respiratory chain complex enzyme activity ¹	Normal values ³	F1/II:2	F2/II-2	F3/II:2
Citrate synthase ²	105-350	186	258	254
I (NADH-decylubiquinone-oxidoreductase)	>15.0	12.9	25.9	17.2
II (Succinate-decylbuniquinone oxidoreductase)	>26.0	50.5	52.1	57.6
III (Decylubiquinol - Cytochrome c oxidoreductase)	>40.0	74.9	77.6	93.2
IV (Cytochrome c oxidase)	>70.0	124	135	118
II + III (Succinate-cytochorme c reductase)	>10.0	22.0	17.7	30.0

Abnormal values are in bold

¹Complex enzyme activity (nmol min⁻¹ mg⁻¹ protein) expressed relative to the citrate synthase specific activity

² Citrate synthase specific activity expressed as nmol min⁻¹ mg⁻¹ protein

³ 2.5th percentile (for citrate synthase, 2.5th - 97.5th percentile range) of 110 controls.

