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CASE REPORT

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The neurological core features of the infantile-onset multisystem neurologic, endocrine, and pancreatic disease: A novel nonsense mutation in an Italian family

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Abstract

Aim: Biallelic mutations in the *PTRH2* gene have been associated with infantile multisystem neurological, endocrine, and pancreatic disease (IMNEPD), a rare autosomal recessive disorder of variable expressivity characterized by global developmental delay, intellectual disability or borderline IQ level, sensorineural hearing loss, ataxia, and pancreatic insufficiency. Various additional features may be included, such as peripheral neuropathy, facial dysmorphism, hypothyroidism, hepatic fibrosis, postnatal microcephaly, cerebellar atrophy, and epilepsy. Here, we report the first Italian family presenting only predominant neurological features.

Methods: Extensive neurological and neurophysiological evaluations have been conducted on the two affected brothers and their healthy mother since 1996. The diagnosis of peripheral neuropathy of probable hereditary origin was confirmed through a sural nerve biopsy. Exome sequencing was performed after the analysis of major neuropathy-associated genes yielded negative results.

Results: Whole-exome sequencing analysis identified the homozygous substitution c.256C>T (p.Gln86Ter) in the *PTRH2* gene in the two siblings. According to American College of Medical Genetics and Genomics (ACMG) guidelines, the variant has been classified as pathogenic.

At 48 years old, the proband's reevaluation confirmed a demyelinating sensorimotor polyneuropathy with bilateral sensorineural hearing loss that had been noted since he was 13. Additionally, drug-resistant epileptic seizures occurred when he was 32 years old. No hepatic or endocrinological signs developed. The younger affected brother, 47 years old, has an overlapping clinical presentation without epilepsy.

Interpretation: Our findings expand the clinical phenotype and further demonstrate the clinical heterogeneity related to *PTRH2* variants. We thereby hope to better

Alessia Mammi and Alessandro Geroldi contributed equally to this study.

define IMNEPD and facilitate the identification and diagnosis of this novel disease entity.

KEYWORDS
IMNEPD, peripheral neuropathy, *PTRH2*, sensorineural hearing loss

1 | BACKGROUND AND AIMS

Infantile-onset multisystem neurologic, endocrine, and pancreatic disease (IMNEPD) (OMIM #616263) is an extremely rare autosomal recessive disorder of variable expressivity caused by biallelic mutations in the peptidyl-tRNA hydrolase 2 (*PTRH2*) gene (NM_001015509. 2), first reported in 2014[1] with only 21 patients described so far in Asia and North Africa[1,2] (Table 1 and Figure 1).

Recurrent features of this disorder include global developmental delay, intellectual disability or borderline IQ level, sensorineural hearing loss, ataxia, peripheral neuropathy, and pancreatic insufficiency (both exocrine and endocrine). Additional features may include facial dysmorphism, hypothyroidism, hepatic fibrosis, postnatal microcephaly, cerebellar atrophy, and epilepsy. Clinical heterogeneity is wide and not all patients present with all features.[1,2] Previous studies in the literature have suggested that missense variants result in less severe phenotypes compared to nonsense variants.[1] Peptidyl-tRNA hydrolase 2 (PTRH2) is an evolutionarily highly conserved protein of the peptidyl transferase RNA hydrolase family located in the outer membrane of mitochondria. *PTRH2* plays a key role in preventing the accumulation of dissociated peptidyl tRNA during protein synthesis, but it has been suggested to perform a variety of secondary functions outside the mitochondria.[1] In particular, upon release into the cytosol, it regulates cell survival and death by promoting caspase-independent apoptosis.[1] The details of how alterations in these pathways lead to multisystemic manifestations in IMNEPD remain to be fully elucidated.[1] Picker-Minh et al. recently suggested that the role of *PTRH2* in Purkinje cell maturation and survival may explain the frequent cerebellar involvement in this disease.[3] Doe et al.[4] show a close interaction between *PTRH2* and α7 integrin in a mouse model, explaining the recurrent muscle weakness. To date, no treatment is available.[1]

2 | CASE REPORT

Here, we report the first Italian siblings carrying a novel biallelic *PTRH2* variant with a remarkable neurological phenotype. The genetic definition was achieved after 30 years of diagnostic odyssey. Moreover, a review of the cases described in the literature reveals a wide spectrum of clinical signs, with peripheral neuropathy being the only one consistently present.

The two brothers, born from consanguineous parents from Southern Italy, were previously reported by Mancardi et al.[5] with a comprehensive clinical, neurophysiological, and neuropathological evaluation. All studies were conducted at the Neurological Clinic of the University of Genoa (Italy) when the affected siblings were 13 and 12 years old, respectively. The family history was negative for neurological disorders and both parents were normal on neurological and electrophysiological examination[5] (Figure 2A). The eldest sibling (IV-1), who is 50 years of age, was diagnosed with psychomotor delay, as assessed by the Brunet-Lezine rating scale, manifested as speech delay and autonomous walking achieved at 18 months.[5] At the age of 3 years and 9 months, muscle atrophy, mild hypoesthesia, and sensory ataxia were present. Additionally, muscle stretch reflexes were absent in the upper and lower limbs and bilateral pes cavus was noted. Following an audiometric test at the age of 6, bilateral sensorineural hearing loss was diagnosed.[5] Nerve conduction studies (NCS) confirmed the presence of demyelinating sensorimotor polyneuropathy 4 years later.[5] He started primary school at the age of 8 and required a special education teacher. The younger brother presented with an overlapping phenotype and underwent the same series of medical examinations.[5] A biopsy of the right sural nerve was performed at 9 years of age and described by Mancardi et al.[5]: the nerve consisted exclusively of small myelinated fibers, while the larger fibers were completely absent. In addition, only a few occasional onion-like formations were observed.[5] Both patients were subsequently followed up at the Regional Epilepsy Centre, "BMM" Great Metropolitan Hospital of Reggio Calabria (Italy). Epilepsy became manifest in the eldest brother at the age of 32 years. Seizures occurred several times a week, lasted 1–2 min, and were clinically characterized by impaired consciousness associated with oral and bimanual automatisms. Seizures were refractory to multiple drug combinations (including carbamazepine, phenobarbital, perampanel, brivaracetam, and lamotrigine). Interictal EEG showed severe left temporal-parietal-occipital epileptic abnormalities (Figure 2B). 3T brain magnetic resonance imaging (MRI) was unremarkable. The last NCS, performed at the age of 40 years, confirmed uniform slowing of ulnar nerve conduction, absence of conduction blocks, and unrecordable sural nerve SNAPs. The younger brother still presents a phenotype that overlaps with that of the older brother, but so far without epilepsy.

Both patients signed an informed consent for genetic testing in accordance with the regional ethics committee and guidelines for genetic testing used in current clinical practice. DNA extracted from leukocytes was first analyzed with a custom CMT-associated gene panel by next-generation sequencing on an Ion Gene Studio S5 System sequencer (Thermofisher). The panel contains the coding and flanking regions of 56 CMT-associated genes. Bioinformatic analysis was performed using Ion Reporter (Thermofisher) and ANNOVAR software. As this initial analysis was negative, whole-exome sequencing was performed in collaboration with IRCCS G. Gaslini in Genoa.

TABLE 1 Clinical characteristics and *PTRH2* variants of all the IMNEPD published patients.

	Khamirani et al., 2021	Parida et al., 2021	Bubshait et al., 2023	Picker-Minh et al., 2016	Sharkia et al., 2017	Alazami et al., 2015	Sharkia et al., 2023	Hu et al., 2014	Ando et al., 2021	Le et al., 2019	Bronson et al., 2021	Our Study
Described individuals	1	1	2 (1 family)	1 + 4 (2 families)	3	1	1	2	1	3 (1 family)	1	2 (1 family)
Ethnic background	Iran	India	Saudi Arabia	Tunisia, Saudi Arabia	Arab[a]	Saudi Arabia	Arab[a]	Turkey	Japan	Syria	India	Italy
Variant	c.68T>C p.Val23Ala	c.127dupA p.Ser43Lysfs*11	c.114dup p.Gly39Trpfs*16	c.254A>C p.Gln85Pro	c.254A>C p.Gln85Pro	c.254A>C p.Gln85Pro	c.254A>C p.Gln85Pro	c.269_270delCT p.Ala90Glyfs*13	c.280T>A p.Tyr94Asn	c.324G>A p.Trp108Ter	c.328G>T p.Glu109Ter[b]	c.256C>T p.Gln85Ter
Variant effect	Missense, minimal damage	Frameshift, LOF	Frameshift, LOF	Missense, LOF	Missense, LOF	Missense, LOF	Missense, LOF	Nonsense, LOF	Missense, LOF	Nonsense, LOF	Nonsense, LOF	Nonsense, LOF
Not neurological signs												
Growth retardation	−	−	+/−	−/+/+/−/−	−/−/−	−	−	+/+	na	+/+/+	+	+/+
Head or face abnormality	−	−	−/−	+/+/−/−/−	+/+/+	−	+	+/+	+	+/+/+	−	−/−
Endocrine involvment	−	+	+/−	+/−/−/−/−	+/+/+	+	na	+/+	+	+/+/+	+	−/−
Liver involvement	−	−	na/na	+/−/−/−/−	−/−/−	−	na	+/−	−	−/−/−	+	−/−
Pancreas involvement	−	−	−/−	+/−/−/+/+	−/−/−	−	+	+/+	+	na	+	−/−
Hand and feet deformities	+	−	+/+		+/+/+	+	+	+/+	+	na	+	+/−
Muscle atrophy/ weakness	+	na	+/+	+/+/+/−/−	+/+/+	+	−	+/+	+	+/+/−	+	+/+
Neurological signs												
Hearing loss	−	+	+/+	+/+/+/+/+	+/+/+	+	+	+/+	+	+/−/−	+	+/+
Motor delay	+	−	+/+	+/+/+/+/+	+/+/+	+	+	+/+	−	+/+/+	+	+/+
Intellectual disability	−	+	+/+	+/+/+/+/+	+/+/+	+	+	+/+	+	+/+/+	+	+/+
Seizures	+ (at birth)	−	+ (1y)/+(1y)	na	−/−/−	−	na	na/na	na	+/+/+	−	+(32y)/−
Ataxia	−	−	na/na	+/+/+/+/−	+/+/+	+	na	+/+	+	+/+/+	+	+/+

(Continues)

TABLE 1 (Continued)

	Khamirani et al., 2021	Parida et al., 2021	Bubshait et al., 2023	Picker-Minh et al., 2016	Sharkia et al., 2017	Alazami et al., 2015	Sharkia et al., 2023	Hu et al., 2014	Ando et al., 2021	Le et al., 2019	Bronson et al., 2021	Our Study
Cerebellar atrophy	−	−	+/na	−/−/+/−/−	+/+/+	na	na	+/+	+	+/+/−	+	−/na
Peripheral neuropathy	−	+	+/+	+/na/na/na/na	+/+/+	+	+	+/+	+	+/+/+	+ axonal	+/+

Abbreviation: IMNEPD, infantile multisystem neurological, endocrine, and pancreatic disease.
[a]Arab community in Israel.
[b]In Bronson et al., p.E110*, + KIF1A: c.223C>T p.R75W.

The exome analysis revealed a novel substitution of the C nucleotide for the T nucleotide at position 256 of the coding sequence of *PTRH2* (c.256C>T NM_001015509) in the homozygous state in both patients and in the heterozygous state in the mother (Figure 2C). DNA from the father was not available. This variant resulted in a premature stop codon at position 86 of the protein (p.Gln86Ter), truncating the protein by 93 residues. It is located in a highly conserved region of the protein and may cause degradation of the mRNA by a nonsense-mediated decay mechanism or produce a truncated, non-functional protein that is likely to be degraded. The presence of this variant was confirmed by Sanger sequencing. The c.256C>T variant of the *PTRH2* gene is reported as pathogenic in the Genome Aggregation Databases (GnomAD) and in the international registry of mutations published in the literature (ClinVar ID: 451081). It was also classified as pathogenic by the ACMG/AMP and ACGS guidelines.[6]

3 | INTERPRETATION

This report depicts a peculiar family in which the genetic diagnosis was achieved more than 30 years following the first neuropathological description. The neuropathological findings reported at that time were different from those described in other demyelinating neuropathies such as CMT1A. The patient's sural nerve consists exclusively of small myelinated fibers, while the larger fibers are completely absent. There is no evidence of demyelination and remyelination, and only a few occasional bulbs are present. The presence of only small, myelinated fibers may be consistent with a very early defect in myelination or with peripheral nerve maturation processes caused by loss of function of the truncating protein. Early damage to myelination could also explain the onset of neuropathy in the two patients described here. Both brothers described here had early-onset progressive motor and sensorimotor polyneuropathy associated with bilateral sensorineural hearing loss, postnatal failure to thrive, delayed motor milestones, and intellectual disability, without additional features. Le et al. described three siblings who developed epilepsy at 7 and 16 years of age treated pharmacologically.[7] Bubshait recently reported two siblings aged 17 and 18 years with generalized cerebral dysfunction on anti-seizure medications.[2] Khamirani described a patient of postnatal/2-day-old epilepsy that resolved spontaneously after 1 year. In all these reports, epilepsy was mentioned as a possible additional feature in childhood or at least in adolescence. Interestingly, our patient had refractory epilepsy that started in adulthood with normal MRI, unlike the previously reported cases. Ataxia is another recurrent feature[1] (Table 1), but as in other cases,[1] no cerebellar involvement was observed in our patients, suggesting a sensory origin secondary to the neuropathy. Our patients had no clinical or laboratory abnormalities suggestive of exocrine or endocrine pancreatic dysfunction, hypothyroidism, or hepatic impairment. Currently, at 32 years of follow-up, the eldest brother was found to have a hepatic angioma at the age of 32 years. There were no other events of clinical significance. No other additional features manifested.

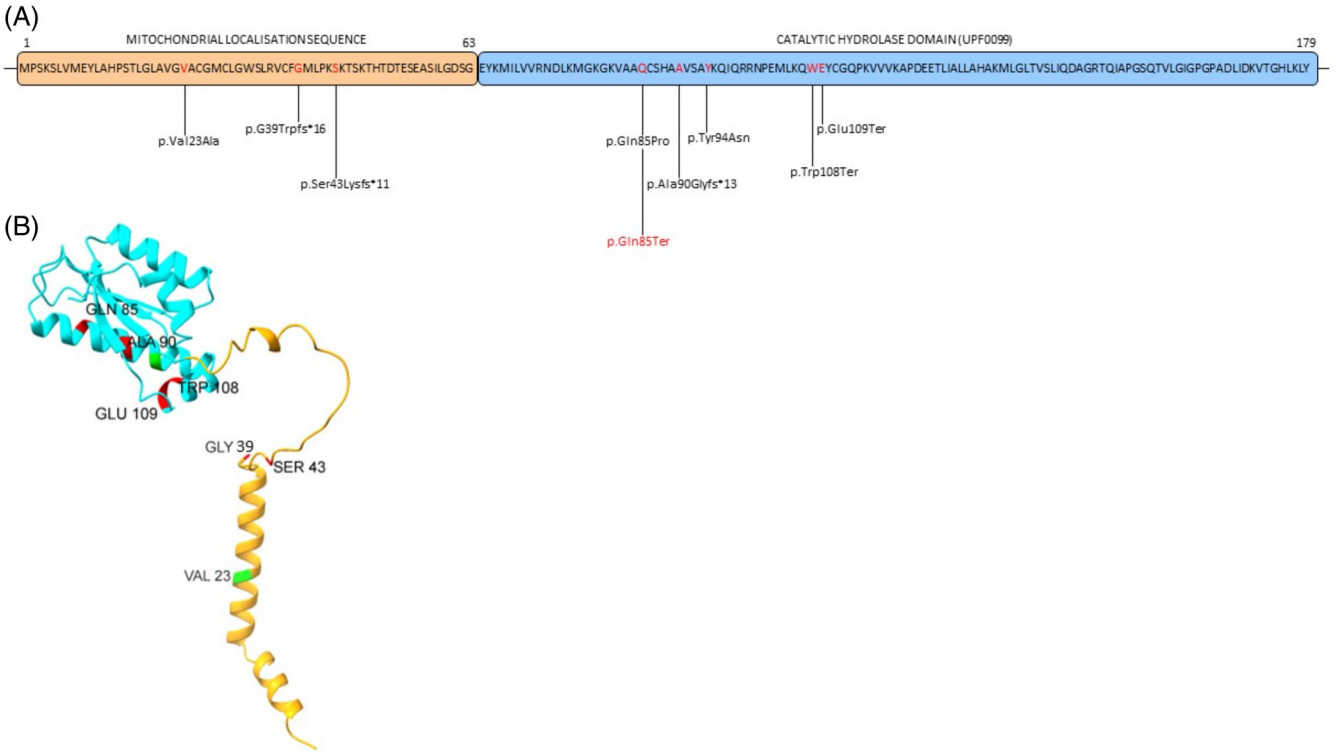

FIGURE 1 (A) Schematic representation of PTRH2 protein: the mitochondrial domain (amino acids 1–61) is depicted in orange, and the catalytic domain (amino acids 63–179) in cyan. Pathogenic variants are in black, p.Gln85Ter in red. (B) The 3D predicted Alphafold model (AF-Q9Y3E5-F1) of the PTRH2 protein (Uniprot: Q9Y3E5) is shown. The mitochondrial localization sequence is in orange and the catalytic hydrolase domain (UPF0099) is in cyan. The codons involved in missense variants are in green and the codons involved in nonsense/frameshift variants are in red.

The patients described here are the first reported family of Italian ancestry and the first described in Europe. The frequency of healthy carriers observed in Europe is comparable to that in Asia (data from Gnomad 4.0); this fact and the pedigree analysis allow us to speculate that the higher disease frequency observed in Asia is due to consanguineous marriages rather than founder effects (at least possible for Q85P in Saudi Arabia). The clinical and neurophysiological characteristics of our patients, compared with literature data, confirm the clinical heterogeneity associated with *PTRH2* variants. Table 1 summarizes the clinical features of all cases described so far. It would appear that there is a very high clinical heterogeneity, further complicated by the fact that the PTRH2 protein is located in the mitochondria. This means that it might have a similar effect to other mitochondrial proteins such as MFN2. The type and positional characteristics of the *PTRH2* variants do not appear to be associated with the disease severity in consideration of the number of additional features, thus making a genotype–phenotype correlation difficult to ascertain. At the molecular level, missense, frameshift, and nonsense variants are likely to cause loss of function anyway. It can be assumed that the occurrence of additional features, such as liver or pancreatic involvement, facial dysmorphisms, cerebellar atrophy, and so on, are not related to the mutation type.

This hypothesis is supported by the observation that clinical heterogeneity is also present in patients belonging to the same family or in patients belonging to different families but sharing the same variant. This behavior is recurrent in many other genetic disorders, but it is particularly marked in mitochondria-related pathologies. Despite the ubiquitous expression of PTRH2, central and peripheral nervous systems seem to be the tissues most affected by the disease (Table 1). Interestingly, if we compare the phenotype of our patients with cases already described in the literature, the core features present in all patients include peripheral neuropathy, bilateral neurosensory hearing loss, motor delay and intellectual disability, although to a greater or lesser extent (Table 1).

Only Khamirani et al. in 2021 described a case without neurological signs. However, the very peculiar phenotype and the predicted minimal damage due to the variant make this patient very difficult to compare to other reported cases. The cerebellar involvement is also recurrent. Picker-Minh et al. demonstrated the central role of *PTRH2* in cerebellar development in mouse models.[3] Interestingly, despite this critical function, not all IMNEPD patients show cerebellar atrophy on MRI. However, to date, no follow-up studies have been conducted to confirm this observation. Muscle tissue is often affected, but probably secondary to the neuropathic damage. The only case with exclusive muscle involvement is that of Kharminani et al.,[1] with the doubts raised above. Despite these considerations, Doe et al. generated a *PTRH2* knockout mouse model that developed a phenotype similar to muscular dystrophy.[4]

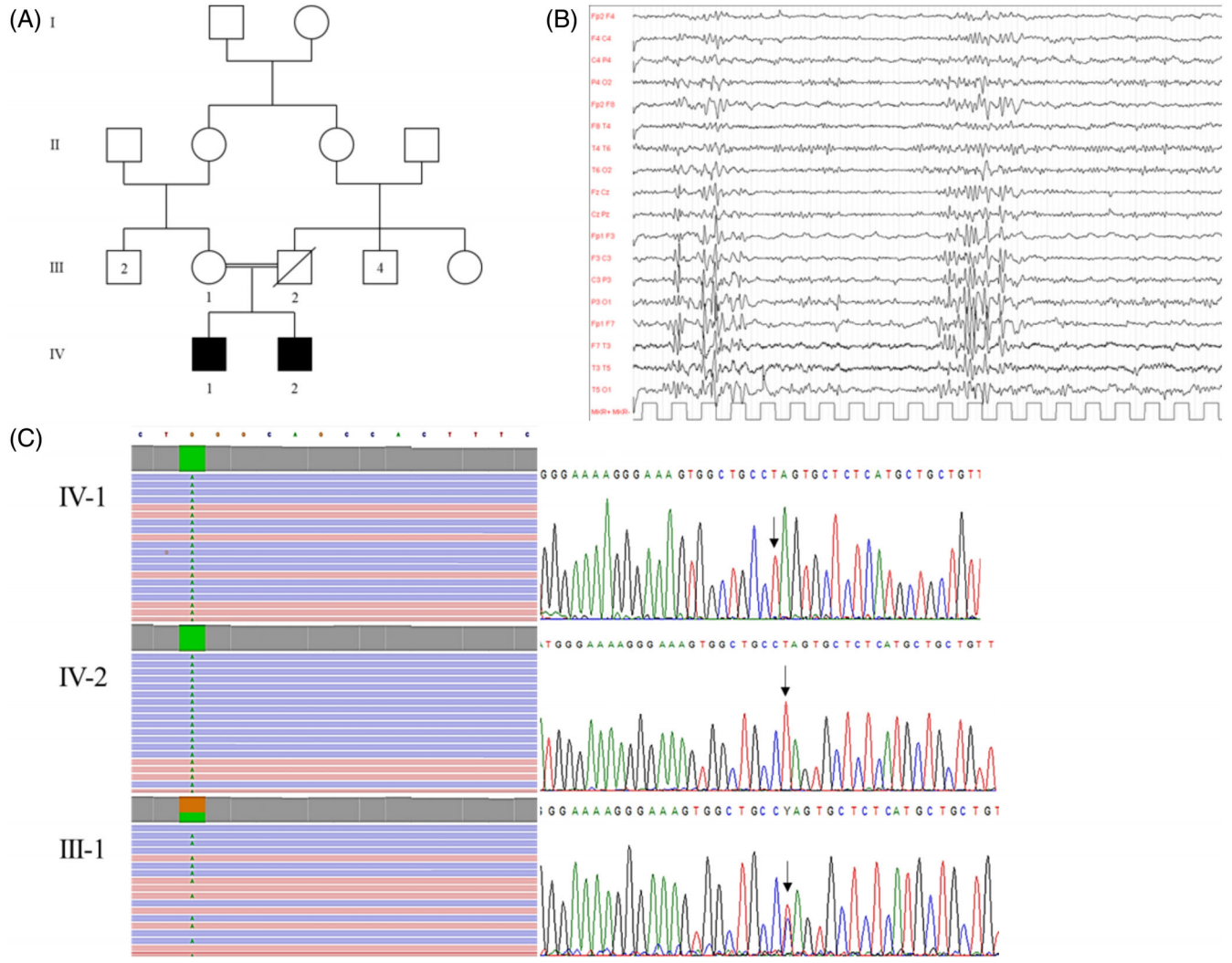

FIGURE 2 (A) Family pedigree. (B) Electroencephalographic recording showing frequent spikes and spike-wave discharges over left temporal-parietal-occipital regions. (C) Integrative genomics viewer and electropherogram of the family members.

In conclusion, our case further expands the spectrum of *PTRH2*-related pathology and suggests that it is a complex neuropathy with possible, but not mandatory, multisystemic involvement. In fact, in our patients, the liver and the pancreas functions are still preserved during 30 years of follow-up. In view of the prevalence of this phenomenon and a paucity of comparable observations in the literature, the occurrence of hepatoangioma described in the elder brother appears to be an isolated event not related to the *PTRH2* variant.

Our findings suggest that *PTRH2* genetic testing should be considered in the molecular diagnosis of hereditary neuropathies, regardless of severity and/or presence of endocrine and/or liver involvement, but also in hereditary hearing loss, especially in association with peripheral neuropathy.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

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