CLINICAL REPORT

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Homozygous mutation in *PTRH2* gene causes progressive sensorineural deafness and peripheral neuropathy

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PTRH2 is an evolutionarily highly conserved mitochondrial protein that belongs to a family of peptidyl-tRNA hydrolases. Recently, patients from two consanguineous families with mutations in the *PTRH2* gene were reported. Global developmental delay associated with microcephaly, growth retardation, progressive ataxia, distal muscle weakness with ankle contractures, demyelinating sensorimotor neuropathy, and sensorineural hearing loss were present in all patients, while facial dysmorphism with widely spaced eyes, exotropia, thin upper lip, proximally placed thumbs, and deformities of the fingers and toes were present in some individuals. Here, we report a new family with three siblings affected by sensorineural hearing loss and peripheral neuropathy. Autozygosity mapping followed by exome sequencing identified a previously reported homozygous missense mutation in *PTRH2* (c.254A>C; p.(Gln85Pro)). Sanger sequencing confirmed that the variant segregated with the phenotype. In contrast to the previously reported patient, the affected siblings had normal intelligence, milder microcephaly, delayed puberty, myopia, and moderate insensitivity to pain. Our findings expand the clinical phenotype and further demonstrate the clinical heterogeneity related to PTRH2 variants.

KEYWORDS

peripheral neuropathy, *PTRH2* gene, sensorineural hearing loss

1 | INTRODUCTION

PTRH2, also known as BIT1 (Bcl-2 inhibitor of transcription 1), is an evolutionarily highly conserved protein that belongs to a family of

peptidyl tRNA hydrolases. It prevents the accumulation of prematurely dissociated peptidyl-tRNA, which could inhibit protein synthesis and be toxic to cells (Sharma et al., 2014; Yao et al., 2014). *PTRH2* is located in the mitochondria where it participates in protein synthesis

via its hydrolase activity. In addition, *PTRH2* was suggested to perform a variety of moonlighting functions outside the mitochondria. For example, in yeast, *PTRH2* inhibits the ubiquitin–proteasome pathway via its interaction with ubiquitin-like (UBL) and ubiquitin associated (UBA) proteins. In particular, an interaction of *PTRH2* with Rad23 and Dsk2, as well as with polyubiquitinated proteins, has been demonstrated. Overall, it was concluded that the UBL-UBA shuttling pathway in the ubiquitin-proteasome pathway is negatively regulated by *PTRH2* (Ishii, Funakoshi, & Kobayashi, 2006). Notably, in mammalian cells, two additional functions of *PTRH2* were reported: (1) Promoting cell survival via activation of both the PI3K-AKT-NFkB pathway and of Bcl-2 transcription (Griffiths et al., 2015, 2011); and (2) Promoting cell death by induction of anoikis (cell death induced upon cell detachment) (Kairouz-Wahbe et al., 2008), which requires release of the protein from mitochondria and formation of a complex with the transcriptional regulator amino-terminal enhancer of split (AES). Thus, PTRH2 is a multifunctional protein that participates in various cellular functions in various locations in the cell (Jan et al., 2004).

Recently, two reports have described families with biallelic variants in *PTRH2* (Alazami et al., 2015; Hu et al., 2014). Alazami et al. (2015) reported two Saudi brothers born to a consanguineous family, with global developmental delay, hypotonia, hyporeflexia, sensorineural hearing loss, ataxia, and clubfoot. One boy had an undescended testicle. The disease-causing mutation was identified as p.(Gln85Pro) in *PTRH2*. The other report described two affected siblings born to consanguineous Turkish parents with a progressive multisystem disease including intellectual disability, postnatal microcephaly, growth retardation, progressive ataxia, distal muscle weakness, peripheral demyelinating sensorimotor neuropathy, sensorineural deafness, exocrine pancreas insufficiency, hypothyroidism, and signs of liver fibrosis. The disease-causing mutation in this study was identified to be a frameshift mutation of *PTRH2* predicted to result in a 78-amino acid truncation. However, the protein was not detected, probably due to instability (Hu et al., 2014).

Here, we report a family in which three siblings harbor a homozygous p.(Gln85Pro) mutation in *PTRH2*, which is similar to the variant that has been previously reported by Alazami et al. (2015). The clinical manifestations resemble those of the previously described patients, but expand the clinical phenotype to include additional, unreported features.

2 | CLINICAL REPORT

The family from the Arab community in Israel was referred to the child development and pediatric neurology clinic due to progressive sensorineural hearing loss and peripheral neuropathy. The parents are first cousins with one healthy son and three affected daughters (Figure 1A).

The eldest affected sibling is a 17-year-old girl who was born at full term by cesarean section, with normal birth parameters, including weight 3.8 kg (75th centile), length 52 cm (75th centile), and head circumference of 33 cm (5th centile). No abnormal signs were noticed immediately after birth. She started walking at the age of 1 year and 8 months. At the age of 2 years, she was referred to the pediatric neurology clinic because of suspected weakness in the lower extremities. The neurological examination revealed hypotonia of

both legs with reduced tendon reflexes, consistent with a peripheral neuropathy. According to the parents' report, she had an expressive language delay and started to express some words at the age of 2.5 years. At the age of 3 years, she was diagnosed by brainstem evoked response audiometry (BERA) with bilateral moderate to severe progressive sensorineural hearing loss. A neuro-developmental evaluation at the age of 4.5 years found expressive language delay. At this age she could express only a few short sentences. At the age of 6 years the "Movements Assessments Battery for Children, second edition" test for evaluation of gross motor function, Beery test for visual-motor integration, and Beery motor coordination test for evaluation of the fine motor functions were undertaken. She had moderate gross motor delay with gait difficulties due to peripheral neuropathy; she can walk on a flat plane but she cannot climb a vertical ladder or climb steps without assistance. She had difficulties with running, catching, and throwing a ball. She also had grapho-motor difficulties such as writing and drawing, without any cerebellar pathological findings. Her neurocognitive evaluation using the Wechsler Preschool and Primary Scale of Intelligence for neuro-cognitive assessment showed that she had normal intelligence. No behavioral difficulties were noticed or reported. She attended a special education school for children with hearing difficulties. At 10 years of age, she underwent detailed testing, including nerve conduction tests (see Table SI). In summary, these neurophysiologic findings are characteristic of a severe demyelinating axonal polyneuropathy, both sensory and motoric. At 14 years old her head circumference was 51.8 cm (2nd centile, mild microcephaly). Metabolic screening, chromosome analysis by routine karyotype, and brain magnetic resonance imaging (MRI) were normal.

By 17 years, the peripheral motor neuropathy had progressed with muscle atrophy of both the upper and lower extremities, especially of hands and feet (Figure 2a–d), and development of bilateral pes cavus deformity (Figure 2c–d). Furthermore, new manifestations were noticed, such as delayed puberty; the older sister had her first menarche at the age of 17 years, the middle sister had her menarche at the age of 15 years, while the youngest sister, who is 13 years old, has not yet had her first menarche.

The two younger affected siblings are 15- and 13-year-old girls. They were both born at full term with normal birth weights. They exhibited a similar clinical course and manifestations as their elder sister, including myopia and microcephaly, severe motor and sensory peripheral demyelinated neuropathy, mild insensitivity to pain with progressive severity and severe bilateral sensorineural hearing loss.

3 | MATERIALS AND METHODS

This research was approved by the ethics committees of the Triangle Research and Development Center, Israel, the UK NHS (11/H1003/3) and the University of Manchester.

Genome-wide SNP microarray analysis using the Affymetrix Genome-Wide SNP6.0 microarray (Affymetrix, High Wycombe, UK) was carried out on DNA from the three affected individuals and the unaffected sibling. Genotypes were generated using the Birdseed v2
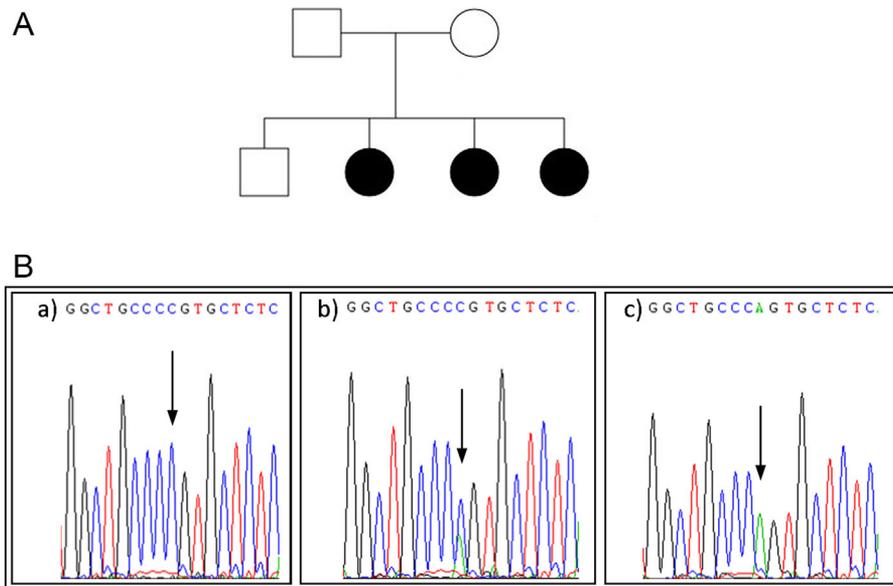
FIGURE 1 (A) Family pedigree: The parents were first-cousin relatives. Squares indicate males and circles indicate females. Black symbols signify affected patients. (B) Electropherogram depicting homozygous missense mutation within *PTRH2* (c.254A>C; p.(Gln85Pro); NM_016077) in the patient (c). The wild-type sequence from a control individual (a) and represents the heterozygous variant present in the parents and unaffected siblings (b) are also shown [Color figure can be viewed at wileyonlinelibrary.com]

algorithm with a confidence threshold of 0.01 within the Affymetrix Genotyping console.

Autozygosity analysis was carried out using AutoSNPa (http://dna. leeds.ac.uk/autosnpa/) (Carr, Flintoff, Taylor, Markham, & Bonthron, 2006). All co-ordinates given are based on hg19. Exome sequencing was carried out for one affected individual using the SureSelect Human All Exon Kit v4 (Agilent Technologies, Edinburgh UK) for the Illumina HiSeq 2500 system (Illumina, Cambridge, UK). Sequence data were mapped to the hg19 reference human genome using the Burrows–Wheeler aligner software (version 0.6.2; http://bio-bwa.sourceforge.net). Genome Analysis Tool Kit software (version 2.4.7; https://www.broadinstitute.

org/gatk) was used for recalibration of base quality score and for indel realignment before using the unified genotyper (https://www. broadinstitute.org/gatk) for variant calling.

Primers were designed for exons predicted to contain a pathogenic sequence variant, as given by the exome sequence data, using Primer 3 (http://frodo.wi.mit.edu/). PCR was performed on genomic DNA using Abgene ReddyMix PCR Mastermix (Thermo-Fisher, Manchester, UK) and sequencing was performed using a BigDye terminator cycle sequencer system v3.1 (ThermoFisher, primer sequences and experimental conditions are available upon request).
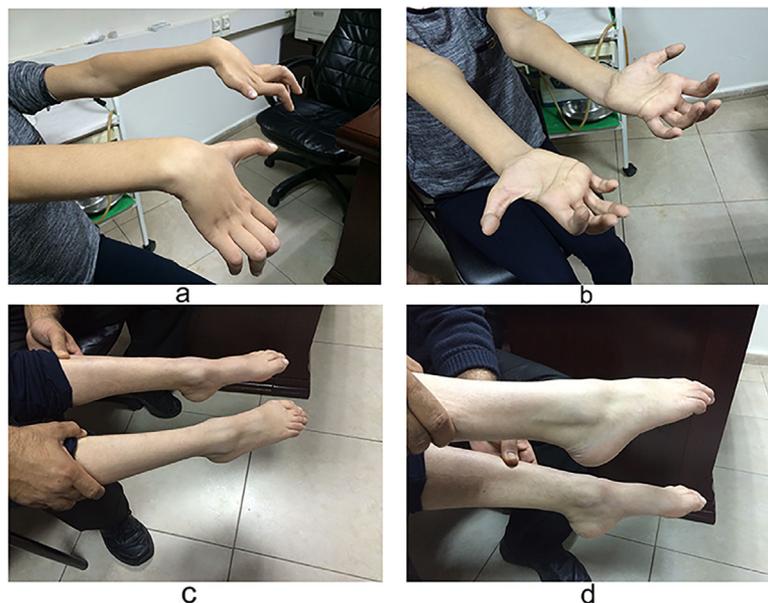

FIGURE 2 (a and b) Long upper extremities with muscle atrophy are readily apparent, especially the muscles of the hands. (c and d) Mild pes cavus deformity of both feet indicates severe peripheral progressive neuropathy [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Clinical manifestations of *PTRH2* disease from the three different studies

Clinical manifestation	Pth2 mutation		
	Our Study	Alazami et al. (2015)	Hu et al. (2014)
	Q85P	Q85P	Truncation by 78 aa
Hearing loss	+++	+	+++
Global developmental delay	−	+	++
Ataxia	−	+	++
Sensory and motor peripheral neuropathy	++	+	++
Mild insensitivity to pain	+	−	−
Microcephaly	+	−	++
Female delayed puberty	+	NA	NA
Growth retardation	−	−	+
Distal muscle weakness	+	+	+
Mild pes-cavus	+	−	−
Clubfoot	−	+	−
Exocrine pancreas insufficiency	−	−	+
Hypothyroidism	−	+	+
Liver fibrosis	−	−	+
Brain MRI	Normal	Arachnoid cyst	Cerebellar atrophy

4 | RESULTS

Five regions of homozygosity >2 Mb were found to be shared by the three affected siblings but not by their unaffected brother. Within the exome sequencing data, 86 homozygous variants were identified within these homozygous regions. After removing variants with a frequency of >1% within the Exome Variant Server (~6,500 individuals, http://evs.gs.washington.edu/EVS/), Exome Aggregation Consortium (>60,000 individuals, http://exac.broadinstitute.org), 1000 Genomes database (http://www.1000genomes.org), or an in-house dataset of over 500 individuals, three variants remained (Table SII). Of these three variants, a missense change within *PTRH2* (c.254A>C; p.(Gln85Pro); NM_016077) was considered most likely to be responsible for the phenotype. The other two rare variants were deemed less likely to result in the phenotype as both were previously identified in healthy individuals, as indicated by analysis of databases of variants identified in control populations. In silico predictions thus provided less compelling evidence of pathogenicity compared to the *PTRH2* variant.

The specific missense variant in *PTRH2* has been previously reported to be associated with similar clinical features (Alazami et al., 2015). In silico analysis predicted that the variant was disease-causing (Mutation Taster 0.99, http://www.mutationtaster.org/; SIFT, score 0, http://sift.jcvi.org/; and Polyphen-2, score 1.0, http://genetics.bwh.harvard.edu/pph2/). The glutamine residue is highly conserved among eukaryotes (Figure S2). Sanger sequencing confirmed that the variant segregated with the phenotype, as the three affected daughters were homozygous for the variant while the unaffected brother and the parents were carriers (Figure 1B).

5 | DISCUSSION

All individuals reported to date with biallelic variants in *PTRH2* have sensorineural hearing loss together with sensory and motor peripheral neuropathy, although to a variable degree (Table 1). Interestingly, the sensory and motor peripheral neuropathy was found to be common to our patients who carry a missense mutation, as well as to the patients carrying the truncating mutation (Alazami et al., 2015; Hu et al., 2014).

Regarding the reproductive system, the male patients in previous reports had an abnormality of the testicles (undescended testes) as well as abnormality of the scrotum. Of note, the females reported here had delayed puberty. The eldest sister had her menarche at the age of 17 years and the middle sister at the age of 15 years, while the mean age of menarche in Israel is about 13 years (Flash-Luzzatti, Weil, Shalev, Oron, & Chodick, 2014). Hu et al. (2014) did not mention pubertal development in the 14-year-old affected female. Therefore, delayed puberty of females could be a feature of disrupted *PTRH2* function.

Although all patients in the three studies were born at full term with normal growth parameters, our patients were not diagnosed with any abnormalities at birth, while the patients reported by Hu et al. (2014) had a progressive multisystem disease that was apparent from birth and included hip dislocation, hypotonia, brachycephaly, mild facial dysmorphism with midface hypoplasia, widely spaced eyes, exotropia, thin upper lip, proximally placed thumbs, and deformities of the fingers and toes. The patients developed postnatal microcephaly, failure to thrive with growth retardation, delayed motor milestones, intellectual disability, progressive ataxia, distal muscle weakness with ankle contractures, demyelinating sensorimotor neuropathy, and sensorineural hearing loss.

Regarding global developmental delay and growth retardation, the differences are clearly evident among the three families. The individuals reported here had expressive language delay and progressive, moderate to severe, gross, and fine motor delay due to progressive sensory and motor neuropathy with insensitivity to pain. In contrast, Alazami et al. (2015) reported that affected individuals had mild global developmental delay, while Hu et al. (2014) reported that his patients had a moderate to severe global developmental delay. While our patients manifest a moderate muscle weakness and moderate gait difficulties, Hu et al. (2014) described

severe clinical manifestations including delayed motor milestones, progressive ataxia, and distal muscle weakness with ankle contractures in their patients. Similarly, while our patients showed mild expressive language delay, Hu et al. (2014) reported that their patients had severe expressive speech delay, but Alazami et al. (2015) did not mention such impairments in his patients. In addition, our patients had normal intelligence, while patients reported by Alazami et al. (2015) had mild intellectual disabilities (IQ = 57), and patients reported by Hu et al. (2014) had moderate to severe intellectual disabilities, with IQs of 39 and 48.

Metabolic abnormalities also varied among patients. Those reported by Hu et al. (2014) had various features, including hypothyroidism, exocrine pancreatic insufficiency with bulky steator-rhea, deficiency of lipophilic vitamins, and abnormal clotting parameters. These symptoms were supported by the evidence of hepatic and pancreatic fibrosis and hepatomegaly using ultrasound. The patients reported by Alazami et al. (2015) had a latent hypothyroidism (TSH 5.01), without any other endocrine or pancreatic insufficiency, whereas our patients did not have any of these features.

While brain MRI showed a normal structure in two of our patients and a left temporal arachnoid cyst, which is a normal variant, in the patients described in the study by Alazami et al. (2015) the patients reported by Hu et al. (2014) had progressive cerebellar atrophy.

In our patients, as well as in those reported by Alazami et al. (2015), a missense mutation in *PTRH2* was found, which correlated with specific clinical manifestations, namely progressive neurosensory hearing loss and peripheral neuropathy. It can be concluded that this type of mutation results in a milder phenotype. On the other hand, the mutation described by Hu et al. (2014) was reported to be a frameshift, thus forming a null allele, and leading to a more severe form of the *PTRH2*-related disorder.

The disease-causing missense mutation in *PTRH2* is found in a strictly conserved glutamine residue, changing it to proline (Figure S2). This high conservation highlights the importance of this amino acid for protein function or for stability of its structure. However, in the three published crystal structures of *PTRH2*, this amino acid was not suggested to be important for catalytic activity. Although both the missense and nonsense mutations have been described in patients, the molecular basis for their effect is still unclear. Thus, it remains for future studies to determine which functions of *PTRH2*, mitochondrial, or cytosolic, are impaired and responsible for the observed phenotypic manifestations. We assume that the clinical spectrum of this new genetic disorder is wider than previously reported. This study expands our knowledge and characterization of the clinical spectrum of this newly recognized condition.

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