European Journal of Neurology

european journal of neurology
the official journal of the european academy of neurology

ORIGINAL ARTICLE OPEN ACCESS

Heterozygous *PNPT1* Variants Cause a Sensory Ataxic Neuropathy

Saif Haddad[1] | Christopher J. Record[1] | Eleanor Self[1] | Mariola Skorupinska[1] | Alexander M. Rossor[1] | Matilde Laura[1] | Gordon Ingle[1,2] | Adnan Manzur[3,4] | Francesco Muntoni[3,4] | Julian C. Blake[1,5] | Mary M. Reilly[1]

[1]Centre for Neuromuscular Diseases, Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, UK | [2]Autonomic Unit, National Hospital for Neurology and Neurosurgery, London, UK | [3]The Dubowitz Neuromuscular Centre, University College London, Great Ormond Street Institute of Child Health & Great Ormond Street Hospital, London, UK | [4]National Institute of Health Research, Great Ormond Street Hospital Biomedical Research Centre, London, UK | [5]Department of Clinical Neurophysiology, Norfolk and Norwich University Hospital, Norwich, UK

Correspondence: Mary M. Reilly (m.reilly@ucl.ac.uk)

Received: 8 November 2024 | **Revised:** 8 January 2025 | **Accepted:** 25 January 2025

Keywords: ataxia | CMT | neurogenetics | sensory ataxic neuropathy | spinocerebellar ataxia

ABSTRACT

Background: Biallelic variants in polyribonucleotide-nucleotidyltransferase-1 (PNPT1) have been associated with a range of phenotypes from syndromic hearing loss to Leigh's syndrome. More recently, heterozygous variants in *PNPT1*, have been reported in three families with cerebellar ataxia and prominent sensory neuropathy.

Methods: Whole genome sequencing was performed in two families with autosomal dominant sensory ataxic neuropathy (SAN).

Results: Segregating heterozygous splice site (c.2014-3C>G) and nonsense (p.Arg715Ter) variants were detected in both families. All patients initially presented with an isolated SAN clinically and neurophysiologically with subsequent variable cerebellar involvement.

Conclusion: We report two heterozygous *PNPT1* variants in two families with a predominant SAN, including the novel p.Arg715Ter. This strengthens the argument of *PNPT1* causing dominant disease and highlights a new cause for dominantly inherited SAN.

1 | Introduction

Inherited neuropathies range from those in which the neuropathy is the sole or predominant feature (Charcot–Marie–Tooth disease), to those in which the neuropathy occurs as part of a multisystem disease (complex inherited neuropathies). With advances in genetic testing, it is increasingly recognised that variants in some genes can produce phenotypic variability, from an isolated neuropathy to a more complex syndrome [1, 2]. Sensory ataxic neuropathies (SAN) are a rare subtype of inherited neuropathy. They are often part of a complex syndrome including cerebellar or mitochondrial features. The genetic causes of a pure or predominant SAN are limited to a small number of genes; expansions in *RFC1* causes CANVAS (cerebellar ataxia, neuropathy and vestibular areflexia syndrome) but can present as a pure SAN; similarly, recessive variants in the nuclear mitochondrial gene *POLG* classically not only causes SANDO (sensory ataxic neuropathy, dysarthria and ophthalmoplegia) but also can present with isolated SAN. Rarely variants in *COX20* and *RNF170* [3–5], reported in only a few families, can cause SAN.

Biallelic variants in polyribonucleotide nucleotidyltransferase-1 (*PNPT1*) have been associated with variable phenotypes ranging from syndromic hearing loss to multisystem Leigh disease. More recently, heterozygous variants in *PNPT1* have been associated with disease. Barbier et al. [6–9] identified two novel splice variants in *PNPT1* in two large families with autosomal dominant sensory and cerebellar ataxia consistent with spinocerebellar ataxia 25 (SCA25), and a further nonsense variant in an individual with SAN and cerebellar signs. Notably, there was

incomplete penetrance and variable expressivity, and other features including cognitive impairment and hearing loss.

PNPT1, a subunit of the exosome complex, encodes PNPase, which localises in the mitochondrial matrix and intermembrane space. It is involved in mtRNA processing and degradation. It has been speculated that disrupted PNPase activity leads to the accumulation of double stranded mitochondrial RNA, which leaks into the cytoplasm and triggers an antiviral signalling pathway [10].

In this study, we report two further families with heterozygous *PNPT1* variants who present with SAN.

2 | Methods

2.1 | Patient Selection and Clinical Assessment

Patients were recruited to the ethically approved 'Charcot-Marie-Tooth Disease and related disorders: A Natural History Study', reviewed by the London Queen Square Research Ethics Committee (REC No. : 09/H0716/61), and signed informed consent forms prior to participation. All participants were clinically assessed in our inherited neuropathy clinic using the validated CMT Neuropathy Score [11].

2.2 | Genetic Analysis

Whole genome sequencing (WGS) was performed as part of the 100,000 Genomes Project (100KGP), following previous negative diagnostic testing (including a minimum of CMT2 [24 genes], hereditary sensory neuropathy [11 genes] and hereditary spastic paraparesis [12 genes] panels) [12, 13]. After negative primary findings reports from the 100KGP (virtual panels were applied including hereditary neuropathy v1.49, hereditary spastic paraplegia v1.205, hearing loss v1.56 and nuclear mitochondrial disorders v1.84), variants were prioritised using our up-to-date in-house virtual gene panel. Candidate variants were confirmed through Sanger sequencing and segregation analysis was performed.

3 | Results

3.1 | Clinical Phenotype

All individuals in Families 1 and 2 presented with a SAN; some individuals developed cerebellar signs. Clinical features are summarised in Table 1. The pedigrees were consistent with an autosomal dominant pattern of inheritance in both families (Figure 1).

3.1.1 | Family 1

The female proband (AIII6) who is now aged 30, had a normal birth but first walked at 17 months old with an unsteady gait. She was diagnosed with scoliosis at the age of 3 years. In the first decade, she developed high arches and was noticeably unsteady. At the age of 10 years, she sustained a relatively painless left ankle fracture followed by multiple toe fractures. During the second decade, the unsteadiness progressed, particularly when in the dark, and she was never able to wear high heels. By the age of 15 years old, she felt numb to above the knees and complained of pins and needles in the feet. Examination at this point revealed a broad-based ataxic gait and she was not able to tandem walk. She could stand on her heels and toes, and Romberg's was positive. Power examination was normal in the upper and lower limbs. She was areflexic throughout. She had a truncal ataxia and mild pseudoathetosis of both upper limbs. Pinprick was reduced to above her wrists and mid-thighs in upper and lower limbs, respectively. Vibration was reduced to the anterior superior iliac spine and was normal in the upper limbs. Joint position was present at the metacarpal-phalangeal joint in the right hand, and normal in the left, and reduced to both knees bilaterally.

She subsequently developed bilateral, low-frequency sensorineural hearing loss requiring a hearing aid at the age of 16 years old. Her ataxia progressed with frequent falls; she used a stick and began using a wheelchair at the age of 25 years old. She began to experience neuropathic pain, unresponsive to amitriptyline, gabapentin, doselupin and lidocaine patches. Over the years, she developed migraine and recurrent vomiting episodes. She attended the emergency department several times due to vomiting, requiring intravenous fluids. These have now resolved. She also developed cerebellar features in her second decade including nystagmus, dysmetria and dysarthria.

The clinical features of the affected family members are presented in Table 1. Her paternal aunt (AII3) not only had a sensory ataxic neuropathy with additional cerebellar signs, but also had striking autonomic features. Her symptoms began in the 3rd decade; she developed numbness in the right hand, followed by altered sensation in the right side of the face and the right leg and, then slowly over the years, extended to the left side. She also experienced neuropathic pain, worst in the feet. She had no weakness but complained of severe unsteadiness which worsened over the years. Concurrently, in her early 30s, she developed severe autonomic symptoms which included severe diarrhoea, urinary urgency and postural hypotension. Extensive investigations excluded an acquired or reversible cause. She had a trial of intravenous immunoglobulin without improvement of either the autonomic symptoms or ataxia. By the age of 47 years, she developed obstructive sleep apnoea requiring a continuous positive airway pressure machine. She died aged 61 years old from unknown cause.

In the other affected family members, the age of onset ranged from the 1st to the 3rd decade. All family members had a pure sensory neuropathy. Her affected paternal grandmother did not have any significant cerebellar features except subtle head titubation. Hearing loss was present in the proband (AIII6) as well as the father (AII4) and brother (AIII7). The proband (AIII6) was more severely affected (as assessed by the CMTNS; score 24) than the father (AII4, CMTNS 5) and the brother (AIII7, CMTNS 14).

Neurophysiology was performed on the proband (AIII6) aged 9 years which showed a severe sensory neuropathy with absent

TABLE 1 | Clinical features.

Patient	AI1	AII3	AII4	AIII6	AIII7	BII6	BIII9
Age at onset	**2nd decade**	**3rd decade**	**1st decade**	**1st decade**	**1st decade**	**2nd decade**	**Infancy**
Age at last examination	85	46	57	30	25	57	22
Phenotype	SAN	Sensory ataxic and autonomic neuropathy	SAN	SAN with cerebellar ataxia	SAN	SAN	SAN worsening with intercurrent infections
Scoliosis	Yes	No	No	Yes	Yes	No	Yes
Pinprick (UL/LL)	Normal	Top of arms/Thighs	Normal/ Knees	Mid forearms/ Upper thighs	Forearms/Calves	Elbows/Waist	Top of arms/ Thighs
Vibration UL/LL	Normal/Costal margin	Right wrists, left normal /Right ankle, left CM	Normal/ Knees	Elbow/Right sternum and left CM	Right shoulder and left MCP/ Sternum	Normal/ASIS	Diminished
Proprioception UL/LL	Normal	Right shoulder, left normal/Ankles	Normal	Shoulders/hip	Left MCP, right normal/Normal	Normal	Diminished
Cerebellar signs	No	Dysmetria	No	Dysmetria, Dysarthria Nystagmus	No	No	Heel shin ataxia
Hearing loss	No	No	Yes	Yes	Yes	No	No
Additional features	Mild head titubation	Abnormal temperature sensation and sweating in torso Diarrhoea Urinary urgency Orthostatic hypotension		Adie's pupils with normal accommodation Recurrent Vomiting +− migraines Mild head titubation		Waddling gait, hyperextended at knees, Extensor plantar	
MRI Brain	ND	Normal	Normal	CA	Mild CA	Normal	CA
CMTNS	ND	ND	5	24	14	15	18

Abbreviations: CA, cerebellar atrophy; CM, costal margin; CMTNS, CMT neuropathy score; MCP, metacarpal phalangeal; ND, not done; SAN, sensory ataxic neuropathy.

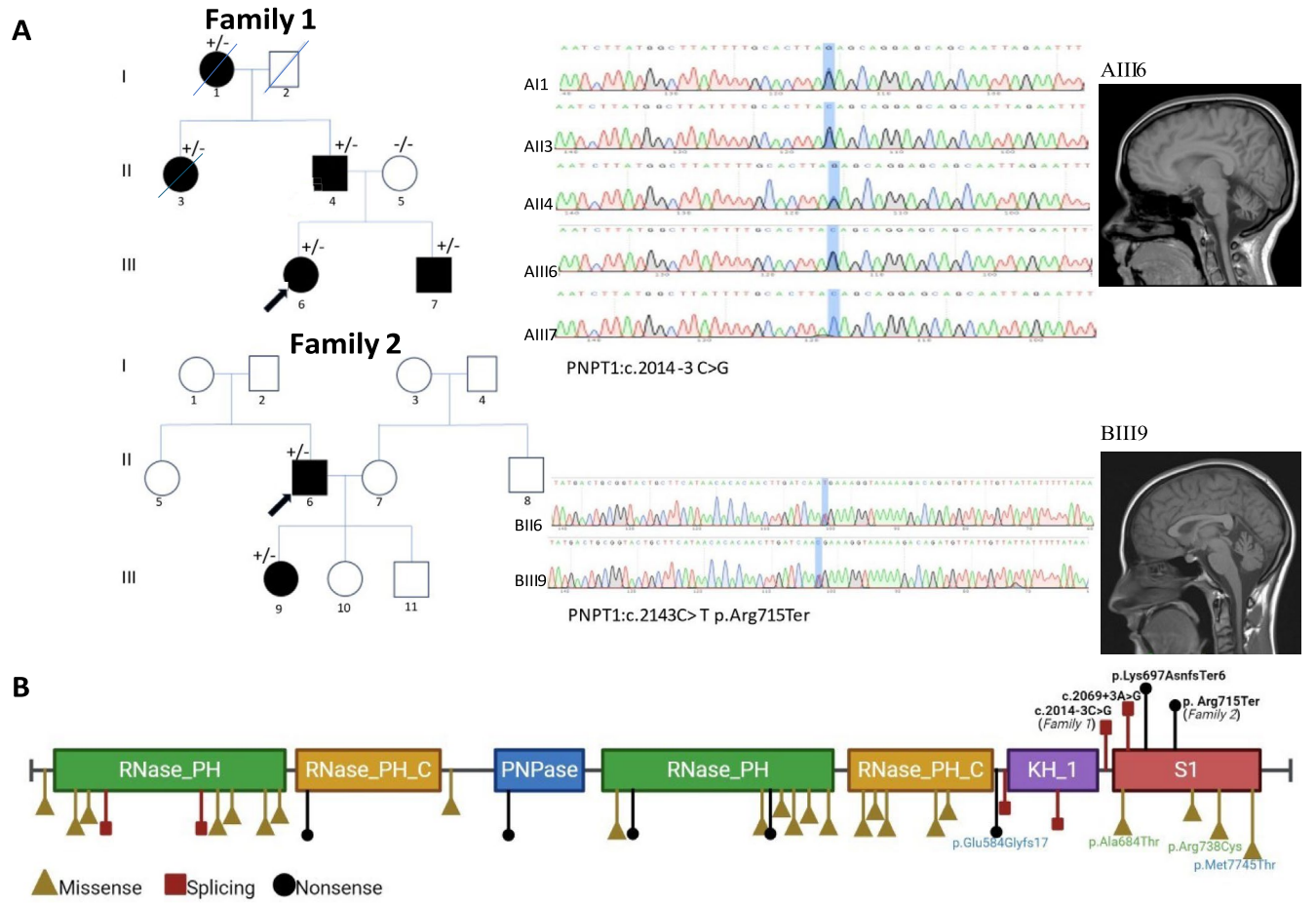

FIGURE 1 | Genetic features of dominant *PNPT1* families A. Pedigrees, Sanger confirmation of heterozygous variants and MRI brain showing cerebellar atrophy (AIII6 and BIII9) of Families 1 and 2. The black arrows represent the probands. B. Schematic of the PNPT1 protein domains and lollipop plot displaying pathogenic variants reported in *PNPT1*. Heterozygous variants are displayed in the above protein schematic, including variants in Families 1 (c.2014–3 C>f the recessive variants, only variants associated with predominant or isolated cerebellar features and/or sensory neuropathy are annotated. Blue/green variants are those found in *trans* in individuals. RNase-PH = 3′exoribonuclease family, domain 1;RNase_PH-C = 3′exoribonuclease family, domain 2:PNPase = polynucleotide nucleotidyltransferase, RNA binding domain; KH_1 = KH domain: S1 = S1 RNA binding domain.

sensory action potentials (SAPs) and a normal motor study right common peroneal compound motor action potential (CMAP) 8.5 mV with a conduction velocity of 48 m/s, (Table S1). Neurophysiology in other affected family members was similar (Table S1). An extensive blood neuropathy screen was normal including transferrin isoforms, biotinidase, vitamin E, porphyrins, very long chain fatty acids, thymidine and deoxyuridine. She had an MRI brain aged 17 years old which revealed cerebellar atrophy (Figure 1). Sequential normal single gene tests included *PMP22* dosage, *FXN*, *SPTLC1*, *SPTLC2*, *RAB7*, *NGF*, *NTRK1*, *POLG*, *ALT1*, *GJB1* and no pathogenic expansions were detected in the genes responsible for SCA 1, 2, 3, 6, 7, 12 and 17. Following negative up-to-date CMT panel (34 genes) testing, the family were subsequently enrolled to the 100KGP.

3.1.2 | Family 2

The proband (BII6) presented in his second decade with a gradual slapping gait over a few years. There was no family history suggestive of a neuropathy. His birth and development were normal. By the age of 23, he had developed balance issues which led to a slow, limping gait with subtle loss of feeling in his feet. He developed hyperextension of his knees and consequently had four arthroscopies in his left knee due to persistent cartilage damage. Over the next decade, he began to fall regularly and suffered a significant left shoulder injury. Despite progression of his unsteadiness, he never complained of weakness. He started wearing foot orthoses in his 30s which helped significantly. His initial examination in his early 40s showed bilateral hyperextension at the knees. He was able to stand on his toes, but not his heels. He had a positive Romberg's test. Cranial nerve examination was normal including fundoscopy. There were no cerebellar signs. He had no finger–nose ataxia or pseudoathetosis. Motor examination including power and tone were completely normal in the upper and lower limbs. Reflexes were absent in the upper limbs, knee reflexes were present and ankle jerks were absent. The plantar response was extensor on the right and equivocal on the left. On sensory examination, pin prick was decreased to the distal calves in the lower limbs but normal in the upper limbs. Vibration was normal in the upper limbs and reduced to the ankles in lower limbs, and proprioception was normal in the

upper and lower limbs. Original neurophysiology performed aged 41 years old showed absent SAPs except a right radial of 5 μV which is absent on a more recent study. His motor study was normal throughout (median CMAP 8.4 mV with a conduction velocity 50 m/s). MRI brain performed aged 41 years old did not show cerebellar atrophy.

His daughter (BIII9) had a normal birth and development. She walked at 14 months with no concerns until 2.5 years of age. She developed an acute illness with pyrexia, drowsiness and unsteadiness. She was treated empirically for encephalitis, although there was no definitive evidence. While she recovered, she remained unsteady, especially while running. At the age of 7 years old, she developed another episode of unsteadiness over a few weeks and was confirmed to have a SAN, without a precipitating illness. Following this episode, she was more unsteady, especially in the dark. She developed scoliosis and had a T3-L4 spinal fusion aged 13 years old. Subsequently, she had another episode of worsening unsteadiness following a possible viral infection associated with severe migraine aged 17 years old. Two weeks following this episode, her examination showed that she walked unsteadily with a positive Romberg's test. Eye movements were normal. She had reduced sensation in V2 and V3 on the left. She had pseudoathetosis of the upper limbs. Tone and power were normal in the upper and lower limbs. Her triceps reflexes were just present, and all other upper and lower limb reflexes were absent. Plantar responses were downgoing. On sensory examination, pinprick was reduced to the shoulders in the upper limbs and to the knees in the lower limbs. There was length-dependent reduction of pinprick across the abdomen. Proprioception was absent in the right upper limb and only present at the shoulder on the left. In the lower limbs, proprioception was reduced to the knees bilaterally. Vibration was reduced in the upper limbs to the sternum bilaterally and in the lower limbs, reduced to the left anterior superior iliac spine and the right sternum. Neurophysiology performed aged 17 years old and aged 22 years old showed absent SAPs with a normal motor study (median CMAP 7.5 mV with a conduction velocity 55 m/s). MRI brain performed aged 17 years old showed cerebellar atrophy (Figure 1).

In summary, the male proband (BII6) clinically had a mild, slowly progressive, sensory ataxic neuropathy with onset in his 20s. His daughter (BIII9) had a more severe SAN, presenting in early childhood, which worsened with intercurrent infections. She developed cerebellar signs at the age of 22.

An extensive neuropathy screen performed on BII6 was normal including vitamin E, copper, amino acid, carnitine profile, porphyrins, lactate and very long chain fatty acids. Urine organic acid was unremarkable. Initial gene testing was negative for *MFN2*, *SPTLC1*, *POLG*, *HSPB1*, *TWNK*, *SOD1*, *RAB7*, *NGF*, *NTRK1*, *NEFL*, *EGR2* and repeat expansion testing in *FXN* and the genes for *SCA* 1, 2, 3, 6 and 7 was negative. He had a normal DNA fragility study, and a negative nuclear mitochondrial gene (21 genes) panel. Whole mitochondrial genome sequencing in muscle was negative. A muscle biopsy showed reduced respiratory complexes II and III activity. White blood cell ubiquinone was normal. Following negative up-to-date CMT panel (24 genes) testing, the family were subsequently enrolled to the 100KGP.

All phenotypic characteristics for both families, including the most recent examination, are summarised in Table 1. Most recent nerve conduction studies are shown in Table S1; a pure sensory neuropathy is demonstrated in all patients (absent SAPs in all patients except AII4 who had small lower limb SAPs with absent upper limb SAPs). All motor studies and EMG, where available, were normal.

3.2 | Genetics

Analysis of WGS variant data through the 100KGP revealed two heterozygous variants in *PNPT1*. Family 1 (AII4, AII5, AIII6 and AIII7) underwent a 'quad' study and the previously reported variant (NM_033109.5 c.2014-3C>G) segregated in all affected members and was absent in unaffected members (Figure 1A). Sanger sequencing confirmed segregation in AI1 and AII3. A novel nonsense variant (NM_033109.5 c.2143C>T p.Arg715Ter) was found in both affected members of Family 2 (Figure 1B). Both variants were absent from gnomAD *V3*. The c.2014-3C>G was absent in gnomAD *V4* and the Arg715Ter has an allele frequency of 1.899×10^{-6}.

4 | Discussion

We report two heterozygous variants in *PNPT1* in two families presenting a predominant SAN, including one novel variant. All patients in our cohort have SAN and some have now developed additional cerebellar and other features. Until recently, biallelic variants in *PNPT1* were known to cause a heterogenous phenotype affecting multiple organs, ranging from non-syndromic hearing loss to multisystemic Leigh syndrome [14–16]. Ruis et al. [17] demonstrated the heterogenous clinical spectrum in 24 patients with biallelic pathogenic variants, the largest case series to date. It revealed that severe developmental delay and regression ($n = 21$) as well as a history of hypotonia ($n = 19$) were the most common abnormalities in the series. Additional common features included sensorineural hearing loss ($n = 13$), optic atrophy and movement and tone abnormalities. Abnormal movements included choreoathetosis, dystonia, myoclonus and ataxia. Other features included autonomic involvement and episodic vomiting.

Heterozygous variants in *PNPT1* have recently been reported to cause cerebellar ataxia and sensory neuropathy. Barbier et al. reported two splice variants, c.2069 + 3A>G and c.2014-3C>G, in large French and Australian families, respectively, with autosomal dominant sensory and cerebellar ataxia. The c.2014-3C>G variant segregating in the Australian family, and in our cohort (Family1), predicted the effect on splicing to be the introduction of a nonsense variant in the S1 domain, p.Gln672SerfsTer6, but RNAseq demonstrated only 10% of the alternate transcript suggesting the majority had undergone nonsense-mediated decay (NMD). This was corroborated by the RNAseq read-depth approximately 50% that of controls, and ~ 50% reduction in PNPase steady state levels. In the French family harbouring c.2069 + 3A>G, it was demonstrated through RT-PCR that the transcript escaped NMD and resulted in skipping of exon 25 and a premature stop codon in the S1 domain (p.Gln672ArgfsTer18). However, steady-state PNPase levels were reduced to around

50% of controls and there was negligible truncated protein seen on western blotting; the authors hypothesised post-translational degradation. Another heterozygous variant p.Lys697AsnfsTer6 consistent with a phenotype of cerebellar ataxia, deafness as well as sensory neuropathy, detected while screening for *PNPT1* in a cohort of 796 French individuals with ataxia, was also studied and, similarly to the c.2069+ 3>G, appeared to escape NMD with RT-PCR showing presence of the aberrant transcript, but reduced PNPase level and negligible truncated protein on western blotting. Therefore, despite some evidence of the variants escaping NMD, the primary mechanism demonstrated through the RNA and protein studies would appear to be loss of function and an intolerance of haploinsufficiency [9].

Although there is an overlap of clinical features between different modes of inheritance, biallelic pathogenic variants tend to show more severe disease, and manifest in the first year of life. Conversely, individuals with an autosomal dominant pattern of inheritance can be affected from the first months of life to 56 years old, implying variable penetrance and expressivity [9]. Notably, in Family 461 reported by Barbier et al., the father of the affected individual carrying p.Lys697AsnfsTer6 was clinically unaffected aged 86 years, and there was an unaffected male carrier in the large French family, although it was not entirely clear whether either individual was examined physically and/or neurophysiologically. Interestingly, previous studies reporting biallelic pathogenic variants had minimal clinical information on the 'healthy' heterozygous parents, and it remains a possibility that heterozygous carriers had a sub-clinical syndrome or have since developed symptoms.

Our cohort confirms the variability previously noted in dominant pedigrees. In Family 1, the age of onset was in the first decade of life in three individuals (AII4, AIII6 and AIII7), whereas one individual (AII3) noticed their first symptom in the 3rd decade. In Family 2, the proband had a later age of onset in the 2nd decade and although was less affected than his daughter who presented at the age of 2 years. Although all patients presented with a sensory ataxic neuropathy, this was non-length dependent in some (AII3, family 1) and the potential multisystem nature of this condition was highlighted in others (AIII6, family 1 developed additional cerebellar involvement, deafness and scoliosis, and BII6, family 2 developed additional pyramidal signs). There was also a wide range of severity from patients being minimally affected (AII4, family 1) to patients being severely affected (AIII6 became wheelchair-bound at age 25).

In conclusion, we report two heterozygous *PNPT1* variants in two families with a predominant SAN, including the novel p.Arg715Ter. The recurrence of the previously reported c.2014-3C>G further strengthens the argument for its pathogenicity. Our report highlights that sensory ataxic neuropathy may be the major and presenting feature in dominant *PNPT1* disease, in contrast with cerebellar ataxia reported previously. The clinical severity is variable, including some apparently asymptomatic adults, and there appears also to be a tight-genotype–phenotype correlation to variants in the S1 protein domain. More work is needed to understand the pathological mechanisms behind these features.

Author Contributions

S.H., C.J.R. and M.M.R. contributed to the conception and design of the study; S.H., C.J.R., E.S., M.S., A.M.R., M.L., F.M., A.M., G.I., J.C.B. and M.M.R. contributed to the acquisition and analysis of data; S.H., C.J.R. and M.M.R. contributed to drafting the text or preparing the figures.

Acknowledgements

MMR and SH are grateful to the Wellcome Trust (G104817). MMR and CJR received funding from the Medical Research Council (MRC MR/S005021/1) and National Institutes of Neurological Diseases and Stroke and office of Rare Diseases (U54NS065712 and 1UOINS109403-01). MMR also receives funding from Muscular Dystrophy Association (MDA510281), Charcot Marie Tooth Association (CMTA), Harrington Discovery Institute, and Alnylam Pharmaceuticals and Applied Therapeutics and is grateful for their support. This research was also supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

1. A. M. Rossor, A. S. Carr, H. Devine, et al., "Peripheral Neuropathy in Complex Inherited Diseases: An Approach to Diagnosis," *Journal of Neurology, Neurosurgery, and Psychiatry* 88, no. 10 (2017): 846–863, https://doi.org/10.1136/jnnp-2016-313960.

2. M. Laurá, M. Pipis, A. M. Rossor, and M. M. Reilly, "Charcot-Marie-Tooth Disease and Related Disorders: An Evolving Landscape," *Current Opinion in Neurology* 32, no. 5 (2019): 641–650, https://doi.org/10.1097/WCO.0000000000000735.

3. A. Cortese, R. Simone, R. Sullivan, et al., "Biallelic Expansion of an Intronic Repeat in RFC1 Is a Common Cause of Late-Onset Ataxia," *Nature Genetics* 51, no. 4 (2019): 649–658, https://doi.org/10.1038/s41588-019-0372-4.

4. M. G. Otero, E. Tiongson, F. Diaz, et al., "Novel Pathogenic *COX20* Variants Causing Dysarthria, Ataxia, and Sensory Neuropathy," *Annals of Clinical and Translational Neurology* 6, no. 1 (2018): 154–160, https://doi.org/10.1002/acn3.661.

5. S. H. Van Daele, M. Moisse, V. Race, et al., "RNF170 Mutation Causes Autosomal Dominant Sensory Ataxia With Variable Pyramidal Involvement," *European Journal of Neurology* 29, no. 1 (2022): 345–349, https://doi.org/10.1111/ene.15091.

6. G. Stevanin, N. Bouslam, S. Thobois, et al., "Spinocerebellar Ataxia With Sensory Neuropathy (SCA25) Maps to Chromosome 2p," *Annals of Neurology* 55, no. 1 (2004): 97–104, https://doi.org/10.1002/ana.10798.

7. S. von Ameln, G. Wang, R. Boulouiz, et al., "A Mutation in PNPT1, Encoding Mitochondrial-RNA-Import Protein PNPase, Causes Hereditary Hearing Loss," *American Journal of Human Genetics* 91, no. 5 (2012): 919–927, https://doi.org/10.1016/j.ajhg.2012.09.002.

8. A. Alodaib, N. Sobreira, W. A. Gold, et al., "Whole-Exome Sequencing Identifies Novel Variants in PNPT1 Causing Oxidative Phosphorylation Defects and Severe Multisystem Disease," *European Journal of*

Human Genetics 25, no. 1 (2017): 79–84, https://doi.org/10.1038/ejhg.2016.128.

9. M. Barbier, M. Bahlo, A. Pennisi, et al., "Heterozygous PNPT1 Variants Cause Spinocerebellar Ataxia Type 25," *Annals of Neurology* 92, no. 1 (2022): 122–137, https://doi.org/10.1002/ana.26366.

10. M. Leszczyniecka, D. C. Kang, D. Sarkar, et al., "Identification and Cloning of Human Polynucleotide Phosphorylase, hPNPase Old-35, in the Context of Terminal Differentiation and Cellular Senescence," *Proceedings of the National Academy of Sciences of the United States of America* 99, no. 26 (2002): 16636–16641, https://doi.org/10.1073/pnas.252643699.

11. S. M. Murphy, D. N. Herrmann, M. P. McDermott, et al., "Reliability of the CMT Neuropathy Score (Second Version) in Charcot-Marie-Tooth Disease," *Journal of the Peripheral Nervous System* 16, no. 3 (2011): 191–198, https://doi.org/10.1111/j.1529-8027.2011.00350.x.

12. D. Smedley, K. R. Smith, and A. Martin, "100,000 Genomes Pilot on Rare-Disease Diagnosis in Health Care—Preliminary Report," *New England Journal of Medicine* 385, no. 20 (2021): 1868–1880, https://doi.org/10.1056/NEJMoa2035790.

13. S. M. Harrison, L. G. Biesecker, and H. L. Rehm, "Overview of Specifications to the ACMG/AMP Variant Interpretation Guidelines," *Current Protocols in Human Genetics* 103, no. 1 (2019): e93, https://doi.org/10.1002/cphg.93.

14. A. Pennisi, A. Rötig, C. J. Roux, et al., "Heterogeneity of PNPT1 Neuroimaging: Mitochondriopathy, Interferonopathy or Both?," *Journal of Medical Genetics* 59, no. 2 (2022): 204–208, https://doi.org/10.1136/jmedgenet-2020-107367.

15. A. Eaton, F. P. Bernier, C. Goedhart, et al., "Is PNPT1-Related Hearing Loss Ever Non-Syndromic? Whole Exome Sequencing of Adult Siblings Expands the Natural History of PNPT1-Related Disorders," *American Journal of Medical Genetics. Part A* 176, no. 11 (2018): 2487–2493, https://doi.org/10.1002/ajmg.a.40516.

16. D. Bamborschke, M. Kreutzer, A. Koy, et al., "PNPT1 Mutations May Cause Aicardi-Goutières-Syndrome," *Brain and Development* 43, no. 2 (2021): 320–324, https://doi.org/10.1016/j.braindev.2020.10.005.

17. R. Rius, N. J. Van Bergen, A. G. Compton, et al., "Clinical Spectrum and Functional Consequences Associated With bi-Allelic Pathogenic *PNPT1* Variants," *Journal of Clinical Medicine* 8, no. 11 (2019): 2020, https://doi.org/10.3390/jcm8112020.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.