

A newly identified Thr99fsX110 mutation in the *PMP22* gene associated with an atypical phenotype of the hereditary neuropathy with liability to pressure palsies

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Hereditary neuropathy with liability to pressure palsies (HNPP) is manifested by a spectrum of phenotypes, from the classical HNPP course associated with intermittent nerve palsies to a neuropathy resembling Charcot-Marie-Tooth type 1 (CMT1) disease. The majority of HNPP cases are associated with submicroscopical deletions in the 17p11.2-p12 region containing the *PMP22* gene, while *PMP22* point mutations are rare, representing about 15% of HNPP cases. In this study, we present a patient manifesting with atypical HNPP phenotype associated with a new Thr99fsX110 mutation in the *PMP22* gene. We conclude that all patients who fulfill the electrophysiological criteria of HNPP, even if they lack the typical HNPP phenotype, should be tested for point mutations in the *PMP22* gene.

Keywords: HNPP neuropathy, *PMP22* gene mutations

INTRODUCTION

Hereditary neuropathy with liability to pressure palsies (HNPP) is a heterogeneous clinical entity that manifests with a wide phenotypic spectrum.

In a large study encompassing 99 individuals affected with HNPP, seven different phenotypes of HNPP have been described.

The majority of patients affected with HNPP present with recurrent peripheral nerve palsies.

A smaller group manifest with a chronic sensory neuropathy. Finally, 20% of patients have been shown to be asymptomatic carriers of the *PMP22* gene deletion (Mouton *et al.*, 1999).

A vast majority of HNPP cases are caused by submicroscopic deletion in the 17p11.2-p12 region encompassing the *PMP22* gene (Chance *et al.*, 1993). In 1993, Nicholson and coworkers reported a first point mutation in the *PMP22* gene that caused

HNPP (Nicholson *et al.*, 1994). To date, only 13 mutations in the *PMP22* gene have been reported causing the HNPP phenotype despite 16 years of systematic studies (*IPNMDB database*: molgen-www.uia.ac.be/CMT Mutations).

In this report, we characterized a patient manifesting with an atypical form of HNPP neuropathy caused by a new Thr99fsX110 mutation in the *PMP22* gene.

Family report

The family came from the south-east part of Poland. The affected family members included the proband, his father and grandfather (Fig. 1a). The proband (IV:10) was born following a normal, uneventful pregnancy. His motor development was normal but according to his father he stumbled and fell frequently. Currently, at the age of 17 years, the

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Abbreviations: CMAP, compound muscle action potential; CMT, Charcot-Marie-Tooth disease; DML, distal motor latency; EMG, electromyography; GJB1, gap junction protein, beta 1; HDA, heteroduplex analysis; HNPP, hereditary neuropathy with liability to pressure palsies; IPNMDB, Inherited Peripheral Neuropathies Mutation Database; MNCV, motor nerve conduction velocity; RFLP, restriction fragment length polymorphism *PMP22*, peripheral myelin protein 22; SNCV, sensory nerve conduction velocity.

proband complains of pain in the legs. Clinical examination at 17 years has revealed no abnormalities except a symmetrical *pes cavus* deformity.

No episodes of peripheral nerve palsies have been observed in the proband until now.

The proband's father (III:9) aged 44 years was completely asymptomatic until the age of 25 years. At that time, he experienced an asymmetrical right foot drop which has not resolved until now.

In neurological examination at the age of 44 years, he manifested symmetrical *pes cavus* deformity that was more pronounced on the right side. He shows gait difficulties with a right foot drop. Ankle reflexes are absent. The patient shows symmetrical muscle wasting in the distal parts of the lower limbs. A mild wasting of the small hand muscles is also visible.

Electrophysiological findings

Neurophysiological studies were carried out on the proband (IV:10) in 2007 and his father (III:9) in 1991. Mixed axonal-demyelinating neuropathy was diagnosed in the proband in the neurographic examination and in the archival EMG examination performed on the father.

In the upper limbs of the proband, compound muscle action potential (CMAP) amplitudes were in the normal range (median 11.4 mV, ulnar 7.4 mV). Motor nerve conduction velocities (MNCV) were slightly reduced (median 44 m/s, ulnar 46 m/s). The latencies in the median and ulnar nerves were prolonged at 6.4 ms and 3.8 ms, respectively.

The sensory nerve conduction velocities in the upper limbs were reduced to 34 m/s in the median and in ulnar nerves.

In the lower limbs, the sural amplitude was slightly reduced (6 μ V) both in the right and left sural nerve. Sensory nerve conduction (SNCV) in the sural nerve was symmetrically reduced (34 m/s).

The superficial peroneal nerve amplitudes were not recordable. MNCV values in the peroneal and tibial nerves were symmetrically reduced to 27 m/s and 32 m/s, respectively. The MNCV values were markedly reduced at the head of fibula (16 m/s and 28 m/s in the right and left peroneal nerves, respectively). EMG showed chronic denervation that was more pronounced in the distal lower limb muscles.

SUBJECTS, MATERIALS AND METHODS

Patients. Approval (No. 120/2008) was obtained from the joint medical and ethics committee at the Warsaw Medical University to perform this clinical and genetic study.

DNA was extracted from blood samples obtained with informed consent from the individuals concerned.

Eighty families were selected from 400 Charcot-Marie-Tooth (CMT) pedigrees for this study on the basis of the absence of the *PMP22* gene duplication/deletion.

High molecular mass DNA was extracted from lymphocytes of 89 CMT affected individuals representing 80 CMT families.

Methods. RFLP analysis was performed using primers, PCR reaction and digestion conditions reported by Stronach and colleagues with some modifications, i.e. PCR amplification for CMT1A and HNPP testing was carried out in 12.5 μ L volumes. From 6 to 10 μ L of amplified products was digested with *EcoRI* according to the manufacturer's instruction (Stronach *et al.*, 1999).

Q-PCR analysis was carried out in conditions previously described by Aarskog and Vedeler (2000) with some modifications. The TaqMan probe for the *PMP22* gene contains 5'FAM fluorophore as a reporter and 3'TAMRA as a quencher and the TaqMan probe for the human serum albumin gene contains 5'VIC as a reporter and 3'TAMRA as a quencher.

The sensitivity of the single strand conformation polymorphism method (SSCP) was tested prior to the screening of DNA samples. Briefly, PCR amplification was performed for DNA samples carrying five mutations previously detected in our laboratory in the *GJB1* gene. In the validation approach, four of these mutations were confirmed.

Five exons of the coding sequence of the *PMP22* gene were amplified by PCR. A standard PCR reaction was carried out in a 25 μ L volume containing: 100 ng of genomic DNA template, 5 mmol dNTPs and 4 pmol of each primer.

PCR was performed for 33 cycles of 1 min at 95°C, 1 min at the respective annealing temperature (exons 1, 61°C; 2, 55°C; 3, 58°C), and 90 s at 72°C.

Both SSCP and heteroduplex analysis (HDA) were performed to screen PCR-products. The PCR products were mixed with SSCP and HDA buffers.

After denaturation for 5 min at 95°C, samples were rapidly cooled on ice.

For HDA analysis, the samples (3 μ L) were mixed with loading buffer (30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol) and denatured for 6 min at 95°C. For renaturation, samples were placed at 65°C for 45 min. The samples were loaded on non-denaturing 6% polyacrylamide gels and electrophoresed in the INGENY

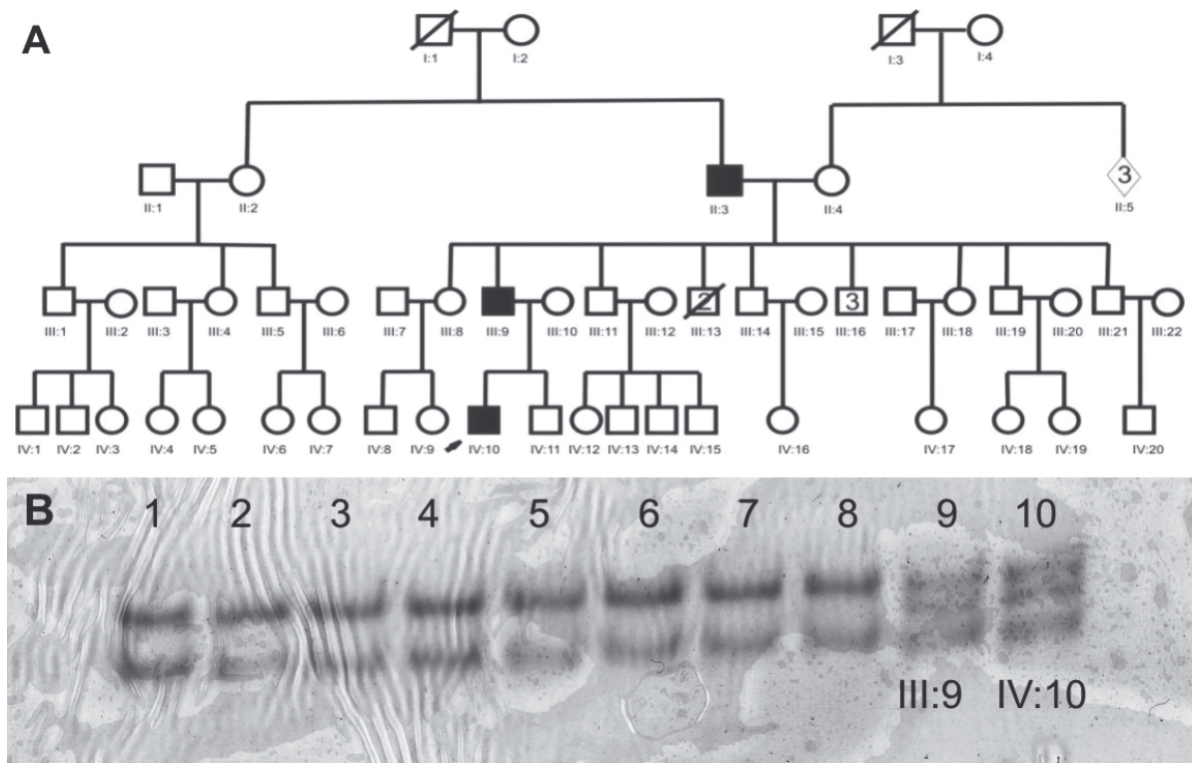


Figure 1. Family tree and results of SSCP screening.

A. Pedigree of the CMT family associated with the new Thr99fsX110 mutation in the *PMP22* gene. The proband is indicated with an arrow. Females are denoted by circles and males by squares. A filled symbol denotes a CMT-affected patient. Deceased individuals are marked with a diagonal line. **B.** Results of SSCP analysis performed for the proband (IV:9), his father (III:10) and healthy controls. Altered SSCP migration was identified due to a heterozygous single nucleotide deletion of T (lanes 9 and 10).

system for 20 h at 150 W. The gels were silver-stained and dried.

Direct sequencing analysis of the PCR products with an altered migration pattern was performed on an 3730/*xl* DNA analyzer using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

RESULTS AND DISCUSSION

Direct sequencing of the *PMP22* gene revealed a heterozygous single nucleotide deletion of T in position 297 (297TΔ), resulting in a frameshift and consequent appearance of a STOP codon at the nucleotide 110 (Thr99fsX110) in the proband (IV:10) and his father (III:9).

Hereditary neuropathy with liability to pressure palsies (HNPP) was described 62 years ago but is still underdiagnosed.

The most likely reason of this relatively low number of HNPP diagnoses may be related to a spectrum of phenotypes that do not fit the classical HNPP course.

The new *PMP22* gene mutation in the patient reported by us was not detected on the ba-

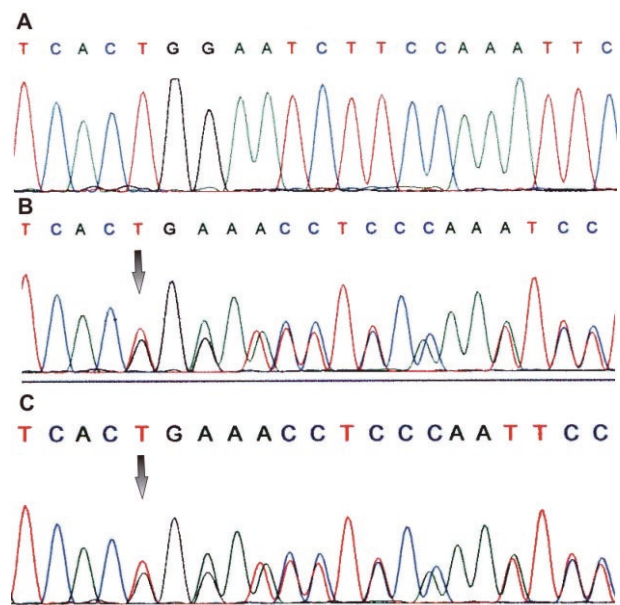


Figure 2. Identification of the 297TΔ (Thr99fsX110) *PMP22* gene mutation.

Direct sequencing revealed a heterozygous one single nucleotide deletion of T at the nucleotide 297 of the *PMP22* gene. **A.** Wild type sequence; **B.** Heterozygous 297TΔ in the proband (IV:10) marked by arrow; **C.** Heterozygous 297TΔ in the father of proband (III:9) marked by arrow.

sis of previous HNPP clinical diagnosis, but was a result of random screening of the *PMP22* gene sequence in 89 CMT-affected patients negative for the *PMP22* gene duplication/deletion.

In fact, the patient reported in our study did not present with clinical features of HNPP, i.e. recurrent painless palsies of the peripheral nerves, but only with a symmetrical *pes cavus* deformity.

In a large comparative study of 70 HNPP-affected patients carrying the *PMP22* gene deletion, only 20% displayed a *pes cavus* deformity (Lenssen *et al.*, 1998).

In contrast to the clinical evaluation, EMG examination revealed three typical features of HNPP delineated by Mouton *et al.* (1999).

The proband showed: (i) increased distal motor latencies in both median nerves, (ii) slowed SNCV in both median nerves across the carpal tunnel in the palm-wrist segment, and (iii) prolonged DML and slowed MNCV in the right peroneal nerve.

Due to a lack of recurrent nerve palsies in the proband, we would rather not classify this entity as HNPP. We propose to describe this phenotype as a mild demyelinating neuropathy associated with the Thr99fsX110 mutation in the *PMP22* gene.

We could not exclude the possibility that some *PMP22* gene variants were not detected in our study. The sensitivity of the SSCP/HDA screening, as validated in our material, is 80%, which means that 20% of *PMP22* gene mutations may have been missed.

However, only 13 mutations in the *PMP22* gene have been detected until now in patients affected with HNPP, and thus it seems unlikely that another mutation could be present in our set of 89 DNA samples.

The pathogenic effect of the Thr99fsX110 mutation seems to be obvious. Nevertheless this mutation was transmitted through three generations in the family reported in our study.

Due to the mild phenotype of CMT caused by the Thr99fsX110 mutation, we suggest a loss of function effect of this variant rather than a dominant negative effect.

Interestingly, the previously reported *PMP22* mutations associated with the CMT phenotype have been postulated to act *via* a dominant negative mechanism. All 13 heterozygous

point mutations in the *PMP22* gene were detected in severely affected patients manifesting with a Dejerine-Sottas phenotype, supporting the hypothesis of the dominant negative effect (IPNMDB database).

Our study further documents the variability of CMT/HNPP phenotypes associated with point mutations of the *PMP22* gene.

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REFERENCES

- Aarskog NK, Vedeler CA (2000) Real-time quantitative polymerase chain reaction. A new method that detects both the peripheral myelin protein 22 duplication in Charcot-Marie-Tooth type 1A disease and the peripheral myelin protein 22 deletion in hereditary neuropathy with liability to pressure palsies. *Hum Genet* **170**: 494–498.
- Chance PF, Alderson MK, Leppig KA, Lensch MW, Matsunami N, Smith B, Swanson PD, Odelberg SJ, Distchele CM, Bird TD (1993) DNA deletion associated with hereditary neuropathy with liability to pressure palsies. *Cell* **72**: 143–151.
- Lenssen PPA, Gabreels-Festen AAWM, Valentijn LJ, Jongen PJ, van Beersum SE, van Engelen BG, van Wensen PJ, Bolhuis PA, Gabreëls FJ, Mariman EC (1998) Hereditary neuropathy with liability to pressure palsies. Phenotypic differences between patients with the common deletion and a *PMP22* frame shift mutation. *Brain* **121**: 1451–1458.
- Mouton P, Tardieu S, Gouider R, Birouk N, Maisonobe T, Dubourg O, Brice A, LeGuern E, Bouche P (1999) Spectrum of clinical and electrophysiologic features in HNPP patients with the 17p11.2 deletion. *Neurology* **52**: 1440–1446.
- Nicholson GA, Valentijn LJ, Cherryson AK, Kennerson ML, Bragg TL, DeKroon RM, Ross DA, Pollard JD, McLeod JG, Bolhuis PA, Baas F (1994) A frame shift mutation in the *PMP22* gene in hereditary neuropathy with liability to pressure palsies. *Nat Genet* **6**: 263–266.
- Stronach EA, Clark C, Bell Ch, Löfgren A, McKay NG, Timmerman V, Van Broeckhoven C, Haites N (1999) Novel PCR-based diagnostic tools for Charcot-Marie-Tooth type 1A and hereditary neuropathy with liability to pressure palsies. *J Peripher Nerv Syst* **4**: 117–122.