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The Thr124Met mutation in the peripheral myelin protein zero (*MPZ*) gene is associated with a clinically distinct Charcot–Marie–Tooth phenotype

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Summary

We observed a missense mutation in the peripheral myelin protein zero gene (*MPZ*, Thr124Met) in seven Charcot–Marie–Tooth (CMT) families and in two isolated CMT patients of Belgian ancestry. Allele-sharing analysis of markers flanking the *MPZ* gene indicated that all patients with the Thr124Met mutation have one common ancestor. The mutation is associated with a clinically distinct phenotype characterized by late onset, marked sensory abnormalities and, in some families, deafness and pupillary abnormalities. Nerve conduction velocities of the motor median nerve vary from <38 m/s to normal values in these patients. Clusters of remyelinating axons in a sural nerve biopsy demonstrate an axonal involvement, with axonal regeneration. Phenotype–genotype correlations in 30 patients with the Thr124Met *MPZ* mutation indicate that, based on nerve conduction velocity criteria, these patients are difficult to classify as CMT1 or CMT2. We therefore conclude that CMT patients with slightly reduced or nearly normal nerve conduction velocity should be screened for *MPZ* mutations, particularly when additional clinical features such as marked sensory disturbances, pupillary abnormalities or deafness are also present.

Keywords: myelin protein zero; Charcot–Marie–Tooth disease; hereditary motor and sensory neuropathies

Abbreviations: bp = base pairs; CMT = Charcot–Marie–Tooth disease; HMSN = hereditary motor and sensory neuropathies; MPZ = myelin protein zero; NCV = nerve conduction velocity; PCR = polymerase chain reaction; SSCP = single-strand conformation polymorphism; STR = short tandem repeat

Introduction

The hereditary motor and sensory neuropathies (HMSN) of the peripheral nervous system represent a group of clinically and genetically heterogeneous disorders (Dyck *et al.*, 1993*a*). The most common types are HMSN type I or Charcot–Marie–Tooth disease type 1 (CMT1) and HMSN type II or CMT2. To date, seven chromosomal locations for autosomal dominant, X-linked and recessive CMT1 have been reported (De Jonghe *et al.*, 1997; Nelis *et al.*, 1998). However, genes for only three autosomal dominant types of CMT1 (CMT1A, CMT1B and CMT1D) and the dominant X-linked form (CMT1X) have been identified (De Jonghe *et al.*, 1997; Warner *et al.*, 1998). CMT1A is associated with mutations in the peripheral myelin protein 22 gene (*PMP22*) on chromosome 17p11.2, and the most frequent mutation is a 1.5 Mb tandem duplication comprising *PMP22*. The less common CMT1B form is caused by mutations in the major peripheral myelin protein zero gene (*MPZ*) on chromosome 1q22-q23. Recently, a dominant mutation in the early growth response 2 gene (*EGR2*) was reported in a CMT1 family (designated here as CMT1D) (Warner *et al.*, 1998). In

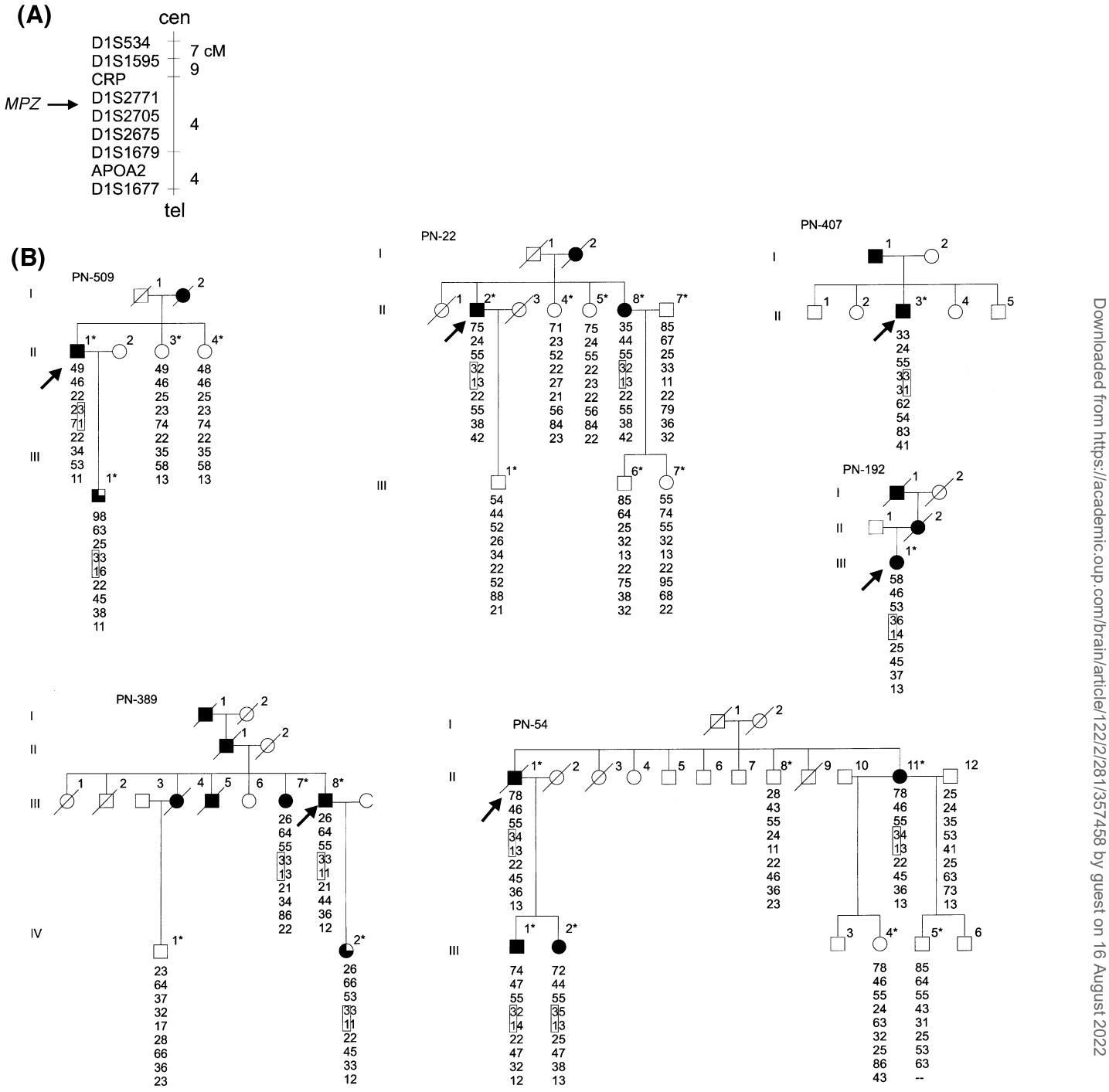
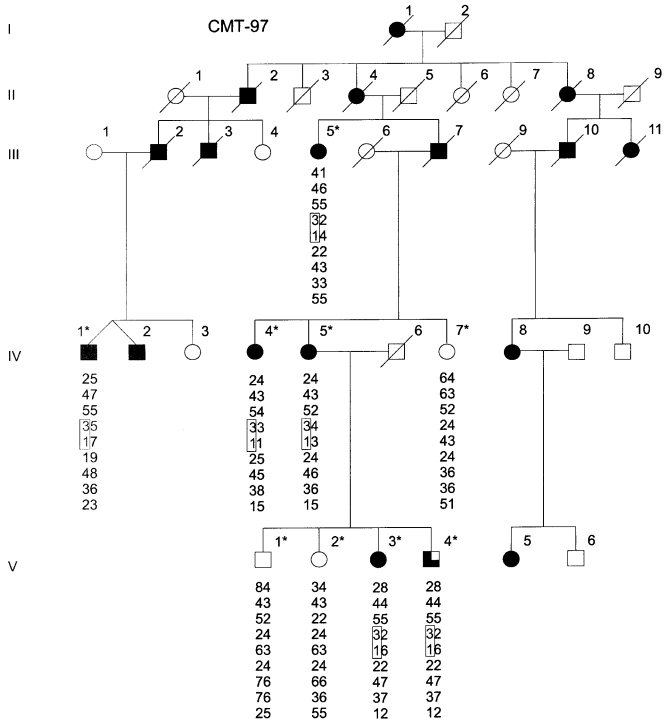


Fig. 1 STR analysis. (**A**) STR markers used for the segregation analysis. cM = centimorgans. Markers D1S2771 and D1S2705 are the flanking markers for *MPZ*. (**B**) Pedigrees of seven Belgian CMT families showing the segregation of STR markers flanking the *MPZ* gene (see above and opposite). Males are represented as squares, females as circles, affected individuals as filled symbols, unaffected individuals and individuals with unknown disease status as empty symbols, and individuals with pupillary abnormalities only as three-quarters-filled symbols. Probands are indicated by an arrow, and individuals clinically examined and sampled for DNA analysis are marked with an asterisk (*); the shared haplotype is boxed.

dominant X-linked CMT1 patients, mutations occur in the connexin 32 (*Cx32*) gene located on chromosome Xq13 (Nelis *et al.*, 1996). Three autosomal dominant CMT2 loci were reported: *CMT2A* on chromosome 1p35-p36 (Ben Othmane *et al.*, 1993), *CMT2B* on 3q13-q22 (Kwon *et al.*, 1995) and *CMT2D* on 7p14 (Ionasescu *et al.*, 1996). No genes have been identified yet for CMT2.

The clinical features of CMT1 are distal muscle weakness starting in the peroneal muscles and eventually resulting in weakness in legs and hands. Foot deformities, such as pes



cavus, are usually present. Subcutaneous nerve trunks are sometimes enlarged. Tendon reflexes are diminished or absent. CMT2 resembles CMT1 clinically, but comparative studies revealed more weakness and atrophy in CMT2 patients, better preservation of tendon reflexes and later onset of symptoms (Harding and Thomas, 1980*a*, *b*). In some families, CMT2 is associated with additional features such as diaphragm and vocal cord paresis (Dyck *et al.*, 1994), deafness or mental retardation (Priest *et al.*, 1995) and mutilating ulcerations (Kwon *et al.*, 1995). Histopathologically, CMT1 patients show extensive segmental de- and remyelination and onion bulb formations in nerve biopsies. CMT2 is characterized by axonal degeneration of the peripheral nerves with relative preservation of the myelin sheaths (Harding and Thomas, 1980*b*; Dyck *et al.*, 1993*b*).

Electrophysiologically, CMT1 is characterized by slowed motor and sensory nerve conduction velocities (NCVs), usually to <38 m/s for the motor median nerve. CMT2 patients have slightly reduced or normal NCVs (Dyck *et al.*, 1993; Harding and Thomas, 19780*b*). However, the study of large X-linked dominant CMT1 families has shown that this CMT type is difficult to classify as either CMT1 or CMT2 based on NCV criteria only. Male CMT1X patients usually have slowed NCVs within the range of CMT1, while female CMT1X patients can have slightly reduced or even normal NCVs within the range of CMT2 (Nicholson and Nash, 1993). Mutation analysis of the *Cx32* gene has shown that these families with a mixture of 'female CMT2 patients' and 'male CMT1 patients' often represent cases of dominant X-linked CMT1 (Timmerman *et al.*, 1996).

Interestingly, distinct point mutations in the *MPZ* gene were observed recently in CMT families with NCVs >38 m/s (Sghirlanzoni *et al.*, 1992; Warner *et al.*, 1996; Marrosu *et al.*, 1998). In this study, we report the phenotype associated with a *MPZ* missense mutation (Thr124Met) in seven Belgian CMT families and in two isolated CMT patients. The patients show variable slowing of NCVs, late age at onset, marked sensory disturbances, pupillary abnormalities and deafness.

Patients and methods

Family data

We previously reported a linkage study in 11 families that we had classified as CMT2 based on motor NCVs (Timmerman *et al.*, 1996). At least one patient in each family had NCVs >38 m/s for the motor median nerve. Re-examination of the NCVs in families CMT-W and CMT-61 showed normal sensory NCVs, and therefore the correct diagnosis in these two families is early onset distal hereditary motor neuropathy type I (distal HMN I). In one family, CMT-28, a Ser49Tyr mutation was found in the *Cx32* gene (Timmerman *et al.*, 1996). The remaining eight CMT2 families (CMT-E, CMT-32, CMT-48, CMT-56, CMT-71, CMT-83, PN-22 and PN-54) have been analysed for linkage to the CMT2A, CMT2B and CMT2D loci on chromosomes 1, 3 and 7, respectively, and negative or non-significant linkage results were found (data not shown). These eight families were selected for mutation screening of the *MPZ* gene. We added to this study seven unrelated Belgian families: four newly diagnosed CMT2 families (PN-192, PN-389, PN-407 and PN-509), one isolated CMT patient (PN-281.1), one CMT family (CMT-97) and one isolated CMT patient (PN-720.1) with pupillary abnormalities (Fig. 1B).

The CMT project was approved by the ethics committee of the University of Antwerp, and informed consent was obtained from all patients and family members participating in the research project.

Neurophysiology and histopathology

Motor and sensory NCV measurements and concentric needle EMG studies were performed according to standard procedures.

A sural nerve biopsy was studied in PN-54 (patient II.1) at the age of 72 years. The nerve was fixed in 4.5% phosphate-buffered glutaraldehyde, post-fixed in 2% phosphate-buffered osmium tetroxide and embedded in Araldite. Semi-thin sections were stained with toluidine blue. Quantitative studies on transverse semi-thin sections of the nerve were done using a Sony camera projecting the picture onto the 21-inch screen of a Macintosh computer. A computer program (Image 1.31 from the National Institutes of Health, Bethesda, Md., USA) was used to determine the number of myelinated fibres per mm^2. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined with a Philips CM10 electron microscope at 60 kV.

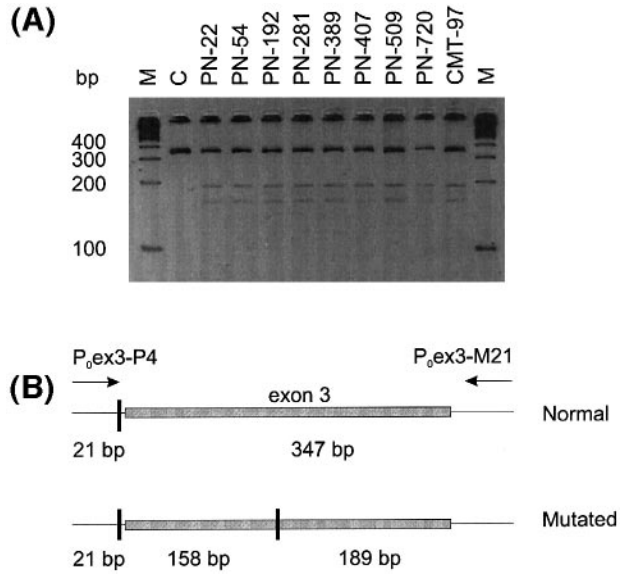


Fig. 2 *MPZ* mutation analysis. (**A**) *Nla*III restriction pattern of the Thr124Met *MPZ* mutation in one affected individual from each family (Fig. 1B) and from the isolated patients PN-281.1 and PN-720.1. In the normal control (C), two restriction fragments were obtained, of 21 bp (not visible under these electrophoretic conditions) and 347 bp. In the affected individuals, the 158 and 189 bp fragments resulted from an additional *Nla*III restriction site created by the Thr124Met mutation. The size marker M represents a 100 bp ladder (Gibco BRL–Life Technologies). (**B**) Normal and mutated *MPZ* PCR products. The vertical bars represent *Nla*III restriction sites. The total length of the *MPZ* PCR product amplified with primers P_0ex3-P4 and P_0ex3-M21 is 368 bp (Nelis *et al.*, 1994*a*, *b*).

Mutation analysis

Single-strand conformation polymorphism (SSCP) analysis was used as a standard procedure to screen for putative mutations in the six exons of *MPZ* (Nelis *et al.*, 1994*b*). The polymerase chain reaction (PCR) samples were subjected to electrophoresis on a 1 × MDE (FMC BioProducts, Rockland, Me., USA) gel at 15 W for 17 h at room temperature and silver staining was carried out according to a standard protocol (Nelis *et al.*, 1997).

Direct sequencing of *MPZ* exon 3 was performed with the intronic primer sequences P_0ex3-P4 (5′-TCATTAGG-GTCCTCTCACATGC-3′) and P_0ex3-M21 (5′-GCCT-GAATAAAGGTCCTTAGGC-3′) (Nelis *et al.*, 1994*b*), where P_0 = MPZ, and the ThermoSequenase Dye Terminator Cycle Sequencing kit (Amersham, Gent, Belgium). The PCR products were loaded onto a polyacrylamide sequencing gel and electrophoresed using an automated DNA sequencer (ABI 377; Applied Biosystems Inc., Foster City, Calif., USA). The data were collected and analysed using the ABI DNA sequencing analysis software, version 2.1.2.

The *MPZ* mutation at codon 124 creates a *Nla*III restriction site that can be detected by restriction digestion of the exon 3 PCR product (Fig. 2). For this purpose, *MPZ* exon 3 was PCR amplified with primers P_0ex3-P4 and P_0ex3-M21 with *Taq* DNA polymerase and PCR buffer (Gibco BRL–Life Technologies, Merelbeke, Belgium). Fifteen microlitres of the PCR amplification product was digested with 10 U of *Nla*III restriction enzyme (New England Biolabs, Hitchin, Herts., UK). The restriction digest was loaded onto a 5% agarose gel (Gibco BRL–Life Technologies) and electrophoresed for 3 h at 120 V. The gel was stained with ethidium bromide.

Haplotype analysis

Genotype analysis was performed with nine short tandem repeat (STR) markers on chromosome 1q22-q23: D1S534 (GATA12A07), D1S1595 (GATA25B02), CRP (C-reactive protein precursor, Mfd57), D1S2771 (AFMb334xb1), D1S2705 (AFMa323xd9), D1S2675 (AFMa244wh5), D1S1679 (GGAA5F09), APOA2 (apolipoprotein A2, Mfd3) and D1S1677 (GGAA22G10). The order of the STRs was deduced from the genetic map of the *Human Co-operative Linkage Center* (http://www.chlc.org/) and the *Whitehead* sequence-tagged site (STS)-based physical map of chromosome 1 (http://www-genome.wi.mit.edu/). D1S2771 and D1S2705 flank the *MPZ* gene on the centromeric and telomeric sides, respectively (Fig. 1A). D1S2705 and *MPZ* are located on the same CEPH YAC 777d5 (590 kilobases) (data not shown). Genomic DNA (0.1 μg) was amplified using oligonucleotide primers labelled with HEX, TET or FAM fluorophores (ISOGEN, Maarsen, Netherlands). PCR was performed in a 25 μl reaction volume containing 10 pmol of each primer and 0.75U *Taq* DNA polymerase (Gibco BRL–Life Technologies). The PCR amplifications were performed in an automated thermal cycler (Techne PHC-3; New Brunswick Scientific, Wezenbeek-Oppem, Belgium). An aliquot of 0.5 μl of each amplified product was mixed with 3 μl formamide, 0.5 μl loading buffer and 0.5 μl fluorescence-labelled ABI size standard GeneScan500-TAMRA (ABI) and heated for 3 min at 95°C. Denatured PCR products were loaded onto 4% polyacrylamide sequencing gels and electrophoresed on the automated DNA sequencer (ABI 37)7. Finally, the data were collected and analysed using GENESCAN version 2.0.2. and GENOTYPER version 2.0 ABI software. The sizes of the alleles were numbered according to the *Genome Data Base* (http://gdbwww.gdb.org/).

Results

Mutation analysis

Subsequent to the report by Marrosu *et al*. (1997) of an *MPZ* mutation (Ser44Phe) in a large Sardinian family diagnosed with CMT2, we screened the eight families (CMT-E, CMT-32, CMT-48, CMT-56, CMT-71, CMT-83, PN-22 and PN-54) as well as five additional Belgian families (CMT-97, PN-192, PN-389, PN-407 and PN-509) and two isolated patients (PN-281.1 and PN-720.1) for mutations in *MPZ* by SSCP analysis. An altered SSCP pattern was detected in all patients

of seven families (PN-22, PN-54, PN-192, PN-389, PN-407, PN-509 and CMT-97) and in the two isolated cases (PN-281.1 and PN-720.1). SSCP analysis of the other five exons of *MPZ* was normal. Direct DNA sequencing of *MPZ* exon 3 revealed a C→T transition at codon 124 (ACG→ATG), resulting in an amino acid change from Thr to Met (data not shown). This mutation creates a *Nla*III restriction site that allows detection of the mutation by PCR amplification of exon 3 followed by *Nla*III digestion (Fig. 2). The Thr124Met mutation was present in all patients of the seven Belgian CMT families and in the two isolated CMT patients. The mutation was also present in the asymptomatic individuals PN-389 IV.2, PN-509 III.1 and CMT-97 V.4 (Fig. 1B). The *MPZ* mutation was absent in 30 normal controls.

Since the same *MPZ* mutation was present in the apparently unrelated families as well as in the two isolated cases and since all patients were of Belgian ancestry, we predicted that they might all be distantly related to a common founder. To test this hypothesis, we analysed several STR markers from the chromosome 1q22-q23 region with D1S2771 and D1S2705, which flank the *MPZ* gene at the centromeric and telomeric side, respectively (Fig. 1A). All familial patients as well as the isolated patients shared one allele at D1S2771 [allele 3 = 257 base pairs (bp), *Genome Data Base* allele frequency, 0.39] and at D1S2705 (allele 1 = 150 bp, allele frequency, 0.30). Haplotype analysis in the families showed that the shared alleles were segregating with the disease haplotype (3 : 1, haplotype frequency, 0.12). Interestingly, some families shared larger disease haplotypes, particularly at the telomeric site of *MPZ*.

Clinical features in patients with the Thr124Met MPZ mutation

Family PN-22 was part of a multigenerational pedigree that had been reported more than 50 years ago (André-van Leeuwen, 1946). Two patients (II.2, II.8) currently 85 and 64 years old, were re-examined for this study. In both patients the disease started in the fourth decade with weakness in the distal parts of the legs, and progressed to complete paralysis. Both patients were wheelchair-bound. They had very severe atrophy of the distal parts of lower and upper extremities and marked sensory abnormalities. Tendon reflexes were absent. Both patients had severe hearing loss which was not observed in unaffected relatives. Pupillary abnormalities had been described in the original report as a peculiar phenotype in this family (André-van Leeuwen, 1946).

In family PN-54 the disease also started in the fourth decade. Patients II.1, II.11 and III.2 developed complete paralysis of the distal parts of the legs and became wheelchair-bound. Distal atrophy and sensory abnormalities in the upper and lower extremities were very marked. Patient III.1 complained of pains and paraesthesias in the lower limbs. He had only slight weakness of the peroneal muscles. His symptoms were attributed to spinal stenosis, for which surgery of the lumbar spine was performed. Patients II.1, II.11 and III.1 had severe hearing loss and patients II.1 and II.11 also had diabetes mellitus. PN-54 II.8 did not carry the Thr124Met mutation but had symptoms and signs of a peripheral neuropathy. However, the disease started later, at the age of 59 years, and the patient was mildly affected, with only sensory abnormalities. This individual also had insulin-dependent diabetes mellitus for 7 years and his clinically different polyneuropathy may be explained by this metabolic disease. We therefore believe that he represents a phenocopy rather than a CMT patient.

The proband III.1 of family PN-192 started to have sensory disturbances in the lower limbs at the age of 37 years. She had bilateral pes cavus, atrophy of the intrinsic hand and feet muscles, and distal paresis in the legs. Pupillary areflexia to light and accommodation was present. Her mother, II.2, was similarly affected, and her maternal grandfather, I.1, had gait disturbances at age of 30 years and became wheelchair-dependent at the age of 40 years.

In family PN-389, the proband III.8 noticed the first symptoms at the age of 46 years. He had distal paresis and atrophy in the lower limbs. Marked sensory abnormalities were observed in the legs and he had sensory ataxia when walking with closed eyes. Pupillary reflexes were normal. Deafness was noticed in the right ear, but this was attributed to a skull fracture. In the proband's father, II.1, a diagnosis of tabes dorsalis was made because of walking difficulties and shooting pains in the legs. The proband's daughter, IV.2, was asymptomatic at the age of 37 years, except for an areflexive mydriatic pupil of the left eye.

The proband in family PN-407 started to complain of paraesthesias in the legs at the age of 41 years. He had slight weakness and atrophy of the peroneal muscles. Sensory disturbances were noted in the feet. His father had walking difficulties and had been examined previously, but the clinical records were no longer available. He was not cooperative for this study.

Patient II.1, from family PN-509, developed walking difficulties at the age of 44 years. Clinical examination at 54 years showed paralysis of the peroneal muscles and weakness of the flexor muscles of the feet. The patient often used a cane to assist walking. Sensibility was markedly diminished in the distal parts of the lower and upper limbs. Bilateral pes cavus was present. His pupils were irregular and unresponsive to light and were therefore diagnosed as Argyll–Robertson pupils. His deceased mother had similar neurological problems and his 18-year-old son (III.1) was asymptomatic apart from weak pupillary reflexes.

In family CMT-97 (PN-280), several patients had a CMT phenotype combined with pupillary abnormalities. The age at onset was later than 30 years. One asymptomatic individual, V.4, had only anisocoria and abnormalities of the pupillary reflexes.

The clinical data from the isolated CMT patient PN281.1 were not available, and patient PN-720.1 was referred for

Table 1 *NCVs in patients with the Thr124Met* MPZ *mutation*

Family and patient	Motor NCV (m/s)			Sensory NCV (m/s)		
	Median	Ulnar	Fibular	Median	Ulnar	Sural
PN-22						
II.2	38 (1400)	26/45 (360/6700)		NR/NR	NR	
II.8	44 (4800)	58 (7000)		48 (5)	NR	
PN-54						
II.1	NR	30 (700)	NR	NR		
II.8	48 (9800)	52 (3100)		41 (3)	NR	
II.11	24/28 (700/600)	39/38 (1900/1300)		NR/NR	NR/NR	
III.1	35 (6300)	52 (2600)	NR	44 (4)	48	
III.2	28/30 (4000/990)	29/26 (7400/4600)		NR	NR	
PN-192						
III.1	32/41 (11900/13000)	47/50 (10800/12000)				
PN-281	30	41				
PN-389						
III.7	47 (11300)	54 (8800)		40 (4)	48	
IV.2	51 (15700)	52 (12400)	45	48 (25)	48	47
PN-407						
II.3	39 (6600)		36			39/41
PN-509						
II.1	39/39 (5500/8200)	50/47 (6400/9000)		47/45 (8/5)	44/41	
III.1	59 (12500)		46	60 (16)		44
CMT-97						
II.2			36			

Amplitudes of evoked responses are given in brackets (for motor NCVs in mV and for sensory NCVs in μV). If the same nerve was measured on both the right and left side, the two values are separated by a forward slash. Normal NCV values: motor median nerve, ⩾49 m/s; motor ulnar nerve, ⩾49 m/s; motor fibular nerve, ⩾41 m/s; sensory median nerve, ⩾46 m/s; sensory ulnar nerve, ⩾46 m/s; sural nerve, ⩾44 m/s. Normal amplitude values: motor median and ulnar nerves, ⩾6000 mV; sensory median nerve, ⩾7 μV. NR = not recordable.

DNA diagnosis as a CMT patient with pupillary abnormalities.

Electrophysiology

The NCVs of patients and asymptomatic mutation carriers are shown in Table 1. The NCVs of the motor median and ulnar nerves ranged from 24 to 59 m/s and from 26 to 58 m/s, respectively (normal values are ⩾49 m/s). The amplitudes of the evoked motor responses were often markedly reduced (normal for our laboratory is ⩾6000 mV for the motor median and ulnar nerves). The lowest NCVs were measured in nerves that innervated severely atrophied muscles. All patients, except PN-54 II.1 and PN-54 III.2, had at least one motor NCV of the median or ulnar nerve that was >38 m/s. Some motor NCVs in the arms were normal. Although patient PN-509 II.1 had normal NCVs for both ulnar nerves, the F-wave latencies were severely slowed to 38–41 ms (normal values are 30–32 ms), pointing to slowed conduction in the proximal part of these nerves. No conduction blocks were observed. His asymptomatic 18-year-old son, PN-509 III.1, had normal NCVs in all nerves tested and the amplitudes of the motor and sensory evoked responses were also normal. The asymptomatic mutation carrier PN-389 IV.2, with only pupillary abnormalities, had normal motor and sensory NCVs in six nerves tested. The concentric needle EMG in this individual was also normal.

Histopathology

A sural nerve biopsy was performed in PN-54 (II.1). On the semi-thin sections (Fig. 3A), loss of myelinated axons was noted, with 3.275 myelinated fibres per mm^2 (normal age-matched values for the sural nerve in our laboratory are 11.434 ± 1.964 per mm^2). Multiple small and large clusters of axonal regeneration were present. Small onion bulbs with one centred myelinated axon and intra-onion bulb regenerating clusters were also seen. In general, myelin sheaths were thin, but normal myelin sheaths as well as occasional thickened myelin sheaths were also observed. No myelin or axonal debris was present. There were no inflammatory infiltrates. Electron microscopy showed onion bulbs composed of one to multiple concentric layers of flattened Schwann cells with or without unmyelinated axons surrounding a myelinated axon or a regenerating cluster. Numerous regenerating clusters, often grouping six well-myelinated axons, were present. Occasional focal myelin thickenings with redundant loop formation were found (Fig. 3B). Some axons were compressed. Normal myelin packing and periodicity were observed. There were signs of denervation of the unmyelinated axons, with empty Schwann cell units and collagen pockets.

Discussion

It has been demonstrated that *MPZ* mutations can lead to peripheral neuropathies that are clinically and

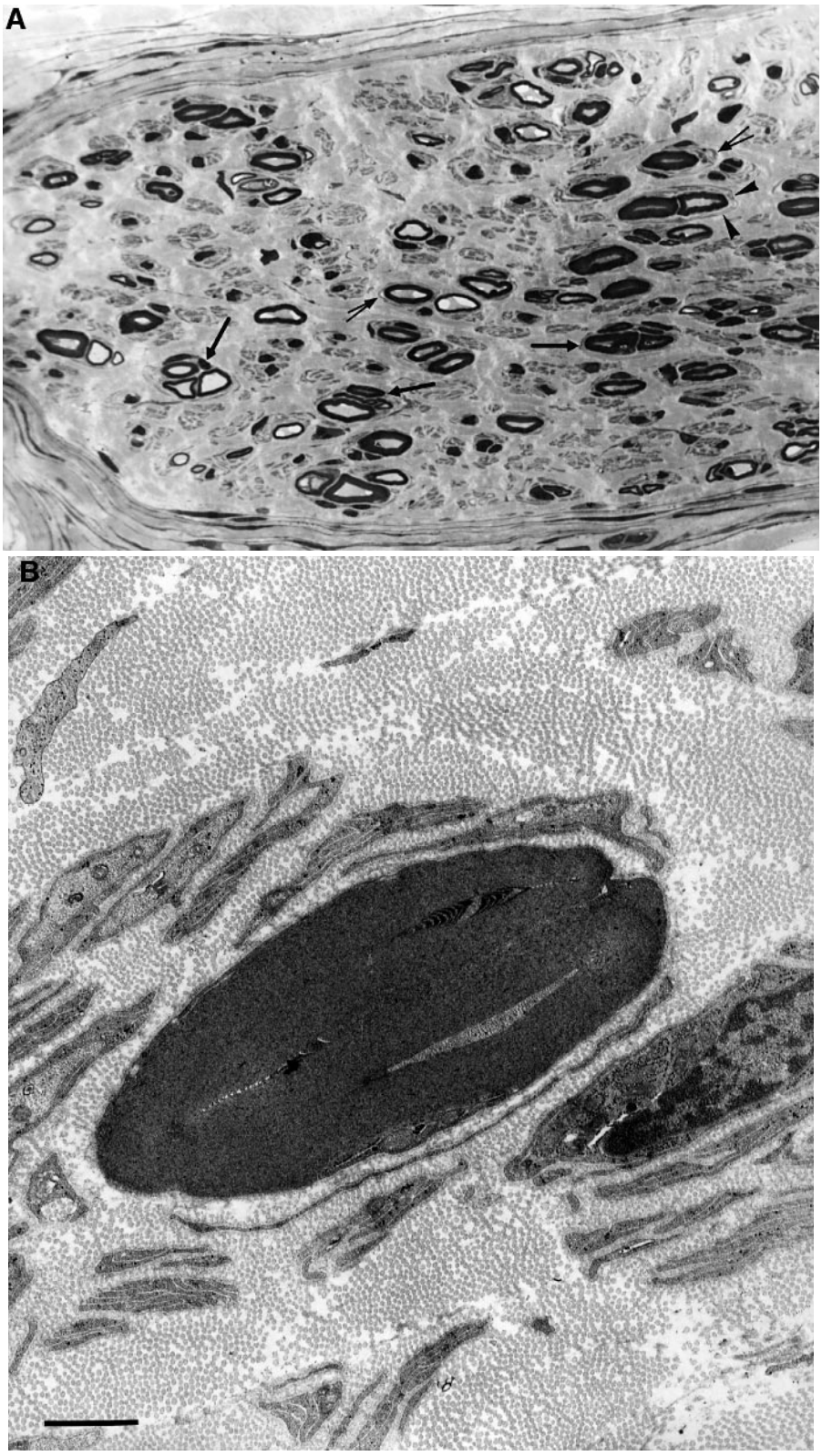


Fig. 3 Sural nerve biopsy of patient II.1 from family PN-54. (**A**) Semi-thin transverse section of the sural nerve showing multiple clusters of axonal regeneration (arrows). Small onion bulbs (open arrows) and intra-onion bulb regenerating clusters (arrowheads) are also seen. Toluidine blue. Magnification: ×637. (**B**) Electron micrograph of a myelin thickening with a redundant loop (tomaculum) wrapping around the entire compressed axon. The structure of the myelin is well preserved. Standard electron microscope techniques (fixation in glutaraldehyde, postfixation in osmium tetroxide, embedding in Araldite, staining with uranyl acetate and lead citrate). Scale bar = 1 μm.

electrophysiologically distinct, such as CMT1, Dejerine–Sottas syndrome and congenital hypomyelinating neuropathy (De Jonghe *et al.*, 1997). The common pathological characteristics of these allelic disorders are severe demyelination and remyelination of peripheral nerves. Electrophysiologically, NCVs in CMT1 patients are severely reduced to <38 m/s for the motor median nerve, and they are even slower or not measurable in Dejerine–Sottas syndrome and congenital hypomyelinating neuropathy. Recently, *MPZ* mutations were also found in CMT patients who had NCVs above the cut-off value of 38 m/s. In a Dejerine–Sottas syndrome family, the sibs of consanguineous parents were homozygous for a frameshift *MPZ* mutation, due to a 1 bp deletion at codon 102 (Warner *et al.*, 1996; Taroni *et al.*, 1996). One of the parents had NCVs as high as 47 m/s for the motor median nerve. The proband had been diagnosed previously with an HMSN III or Dejerine–Sottas syndrome phenotype due to homozygous expression of a 'dominant CMT2 gene' (Sghirlanzoni *et al.*, 1992). Recently, another *MPZ* mutation was found in 16 affected individuals from a large Sardinian CMT2 family (Marrosu *et al.*, 1998). The motor median NCV ranged from 42.8 to 57.3 m/s and in some patients NCVs were not recordable. The mutation was a missense mutation Ser44Phe in the extracellular domain of *MPZ*. Together, these observations suggest that not all *MPZ* mutations result in severely slowed NCVs.

We performed a mutation analysis of *MPZ* in 13 CMT families, of which 12 were classified as CMT2 since at least one patient had NCVs >38 m/s. A novel Thr124Met mutation in exon 3 of the *MPZ* gene was found in 18 patients and three asymptomatic at-risk individuals from seven Belgian families. The same mutation was also found in two isolated Belgian CMT patients who were referred to us for DNA diagnosis of CMT. Haplotype analysis of STR markers flanking the *MPZ* gene demonstrated that these families probably had one common founder, explaining its high frequency in Belgian CMT2 patients. The mutation was not found in 30 normal control individuals or in a large population of CMT1 patients screened for *MPZ* mutations. The Thr124Met mutation was also described recently by Schiavon *et al.* (1998) in a 49-year-old man diagnosed with CMT1, but with late onset of disease (42 years) and decreased motor NCVs (37 m/s). Wolf *et al.* (1997) reported the Thr124Met *MPZ* mutation in one adult patient with a 'moderate' peripheral neuropathy.

All our patients with the Thr124Met mutation had some clinical features in common. The disease usually started in the fourth to fifth decade and progressed rapidly to severe weakness of the lower limbs, many patients eventually becoming wheelchair-bound. Many patients initially complained of lancinating pains in the legs. In the older generations, several patients were diagnosed with tabes dorsalis based on the combination of shooting pains in the legs and pupillary abnormalities. Sensory symptoms are very unusual in hereditary demyelinating neuropathies but are more often observed in acquired neuropathies (Dyck *et al.*, 1993*a*). Severe sensory abnormalities were also detected on routine clinical examination. Patients in families PN-22, PN-54 and PN-509 also had severe hearing loss, and abnormalities of pupil reactivity were observed in families PN-22, PN-509, PN-720.1 and CMT-97. Hearing loss is frequently observed in inherited peripheral neuropathies and is a constant symptom in some types, such as the HMSN found in some Bulgarian gypsy families (HMSNL) (Kalaydjieva *et al.*, 1996). Pupillary abnormalities are sometimes present in other types of inherited peripheral neuropathies, but they were such a constant feature in family PN-22 that more than 50 years ago CMT with pupillary abnormalities had been reported as a distinct clinical entity (André-van Leeuwen, 1946). Three asymptomatic mutation carriers had only pupillary abnormalities. Their normal status can be explained by the fact that they had not yet reached the age of onset for CMT in their families.

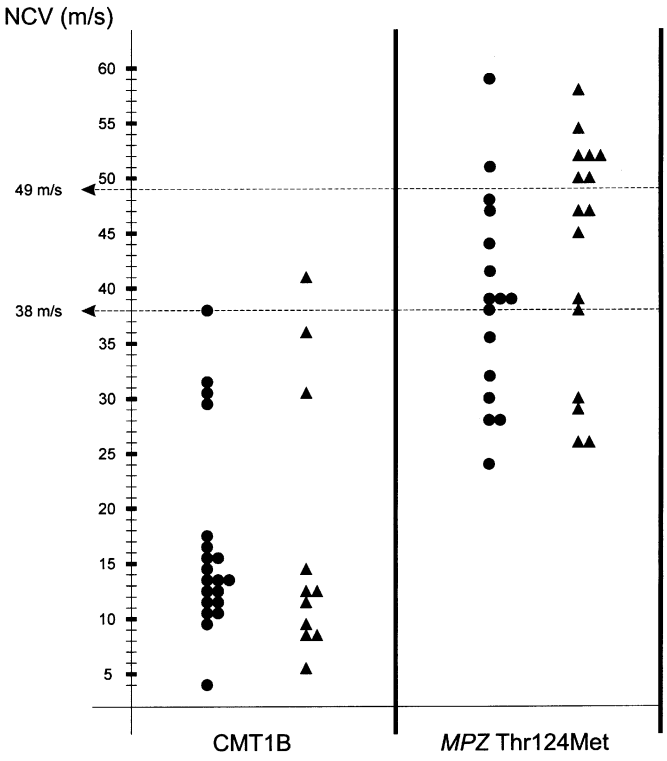


Fig. 4 NCV studies. NCVs of motor median and ulnar nerves in CMT1B with different *MPZ* mutations compared with NCV values measured in patients with the Thr124Met *MPZ* mutation. The 38 m/s cut-off value used to discriminate CMT1 patients and the normal 49 m/s value are indicated by horizontal dotted lines. Closed circles = motor median NCVs; closed triangles = motor ulnar NCVs.

The electrophysiological features were similar in all families with the Thr124Met mutation. The NCVs varied widely and could be severely slowed, as in CMT1, slightly reduced, as in CMT2, or even normal. When the motor NCVs fell within the range of CMT1, i.e. <38 m/s, the values were still higher than those we usually observe in autosomal dominant CMT1 patients carrying the 1.5 Mb CMT1A tandem duplication (data not shown) or a point

mutation in the *MPZ* gene (CMT1B) (Fig. 4). In our CMT1A and CMT1B patient populations nearly 50% of patients had NCVs ranging from 6 to 25 m/s (data not shown). Two asymptomatic mutation carriers, PN-509 III.1 and PN-389 IV.2, had completely normal NCVs. However, all clinically affected individuals had abnormal NCVs in at least some nerves. We do not have serial NCV studies in our patients, but the finding of initially normal NCVs in asymptomatic mutation carriers and severely slowed NCVs in patients suggests that NCV slowing associated with the Thr124Met mutation is a progressive process. The mechanism of NCV slowing in patients with the Thr124Met mutation is probably based on a combination of demyelination and loss of large, fast-conducting axons, as reflected in the severe muscle atrophy and the markedly reduced amplitudes of the motor evoked potentials (Table 1). In CMT1A and most CMT1B families NCVs were homogeneously reduced from birth onwards and remained constant or declined only slightly during ageing. Therefore, in CMT1A and in most CMT1B families NCV slowing is a fully penetrant feature and the detection of slow NCVs is a 100% reliable diagnostic test equivalent to DNA diagnosis. However, in families with the Thr124Met mutation, normal NCVs do not exclude the presence of this particular mutation and DNA testing is essential to determine risk status.

A sural nerve biopsy, performed in one patient (PN-54, II.1) with the Thr124Met mutation in *MPZ*, indicated a process of de- and remyelination with small onion bulbs. However, multiple axonal regenerating clusters revealed an associated axonal involvement with axonal regeneration. In CMT1B patients with other *MPZ* mutations, a classically demyelinating process was observed with onion bulb formation. Uncompacted myelin was found in some cases in 23–68% of the myelinated fibres and in other cases there was abundant occurrence of focally folded myelin or tomaculae (Gabreëls-Festen *et al.*, 1996). Some focally thickened myelin sheaths were also found in the sural nerve of patient II.1 in family PN-54.

In conclusion, our data suggest that the Thr124Met mutation in *MPZ* is a pathogenic mutation associated with a particular clinical, electrophysiological and pathological phenotype. Families with this mutation will often be diagnosed as CMT2 because of their high NCV. Consequently, CMT2 families should be screened for *MPZ* mutations. It is currently unknown how frequent *MPZ* mutations are in the CMT2 patient population. However, we observed the Thr124Met mutation in two out of eight CMT2 families originally selected for linkage studies (Timmerman *et al.*, 1996). The number of different *MPZ* mutations that cause a CMT2-like phenotype remains to be determined. The Thr124Met mutation is located in the extracellular loop of the MPZ protein close to the glycosylation site at codon 122 and the cysteine residue at codon 127, the latter being involved in the formation of a disulphide bridge. It is currently unknown whether and how this mutation disrupts the normal adhesion function of the MPZ molecule, and why the corresponding phenotype differs from what is generally observed in classical CMT1B. Expression studies in cultured cells or transgenic animals will be helpful in determining the effect of this *MPZ* mutation on the function, structure and interactions of the MPZ protein.

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