

Short reports

Mutation analysis of the nerve specific promoter of the peripheral myelin protein 22 gene in CMT1 disease and HNPP

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Abstract

We analysed the nerve specific promoter of the peripheral myelin protein 22 gene (PMP22) in a set of 15 unrelated patients with Charcot-Marie-Tooth type 1 disease (CMT1) and 16 unrelated patients with hereditary neuropathy with liability to pressure palsies (HNPP). In these patients no duplication/deletion nor a mutation in the coding region of the CMT1/HNPP genes was detected. In one autosomal dominant CMT1 patient, we identified a base change in the non-coding exon 1A of PMP22 which, however, did not cosegregate with the disease in the family. This study indicates that mutations in the nerve specific PMP22 promoter and 5' untranslated exon will not be a common genetic cause of CMT1A and HNPP.

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Charcot-Marie-Tooth disease type 1 (CMT1), the major subtype of the hereditary peripheral neuropathies, is characterised by weakness and atrophy of distal limb muscles, diminished or absent deep tendon reflexes, severely slowed motor and sensory nerve conduction velocities (NCV), and de- and remyelination with onion bulb formation on peripheral nerve biopsy.¹ CMT1 is genetically heterogeneous with dominant gene defects on chromosomes 17p11.2, 1q22-23, and Xq13.1. The most common CMT1 mutation is a 1.5 Mb duplication in the chromosomal region 17p11.2 comprising the peripheral myelin protein 22 gene (PMP22).² Furthermore, mutations in the coding regions of the PMP22, myelin protein zero (MPZ), and

connexin 32 (Cx32) genes have been associated with CMT1.³ A few autosomal dominant families do not show linkage to either chromosome 1q22-q23 or 17p11.2 suggesting that there is at least one other locus for autosomal dominant CMT1.⁴

Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominant demyelinating peripheral neuropathy characterised by recurrent nerve palsies resulting from minor trauma. Nerve biopsies show typical sausage-like thickenings.⁵ The majority of HNPP cases are caused by a 1.5 Mb deletion in 17p11.2, the reciprocal mutation to the duplication found in most CMT1 patients. In three cases, HNPP was caused by a PMP22 mutation resulting in an aberrant protein.³ The molecular data indicate that PMP22 is a dosage sensitive gene resulting in CMT1A through gene duplication or gain of function mutations, and resulting in HNPP through gene deletion or mutations leading to an aberrant protein. No mutation was present in the coding regions of PMP22, MPZ, or Cx32 in several non-duplicated CMT1 patients or of PMP22 in several non-deleted HNPP patients. One possibility is that these patients have mutations in as yet unidentified genes.⁴ Another possibility is that the patients have mutations in the non-coding regions of the three known CMT1 genes. Recently, two different point mutations in the non-coding region of Cx32 were identified in two X linked dominant CMT1 families.⁶

We hypothesised that a mutation in the nerve specific PMP22 promoter could mimic the dosage effect by altered expression of the PMP22 gene. Therefore, we analysed the nerve specific promoter region of PMP22 in non-duplicated CMT1 patients and non-deleted

Table 1 Primer sequences for PCR amplification and sequencing of the nerve specific promoter region of PMP22

Primer	Position	Primer sequence (5'→3')	Length of PCR fragment (bp)
Fragment 1	1F	-448→-429	278
	1R	-170→-190	
Fragment 2	2F	-251→-228	332
	2R	81→57	
Fragment 3	3F	-5→16	215
	3R	210→190	

-496 CTTCCAACCAGGGGTTGGAACAAAGGAGGCTTGATTTGGTGACTCTTGAG^{1F-}

-446 ACATTTGGCTCACTGCTGTGATGCTGTGAGAGATTAGCTGTGCAATGTTT

-396 GGGCTCCTTAAAAGGAAGTTTATTTAAATAAAAACCTTACCTGCACGTATG

-346 TAACTACTGTAGACACAGATCCTTCCCACAGTATATTTAATCTCTGCAGAA

-296 TTCACTGGGAGGGGAGGGAGCCAGTGGGACCTCTTGGCTATTAC^{2F-}ACAGG

-246 TTGGCACTTCCAGAGAGAACAGTCTTGGCATCACAGGCTTCAGGCATACT

-196 CAAAGCTCTTCTCCCTTCTGATTCCAGTTTCTCCATGCCTGCAGGGCCT^{-1R}

-146 CTTGGGATTATTGTATTCTGGAAAGCAAACAAAGTTGGACACTGTCTCTT

-96 TAAATAATAGAGGCTGAGAACCCTCAGGCCACCATGACATATCCCAGCA

-46 TTGGACCAGCCCCTGAATAAACTGGAAGACGCCTGGTCTGG^{3F-}CTTCAGTT^{*}

+5 ACAGGGAGCACCACCAGGGAACATCTCGGGAGCCTGGTTGGAAGCTGCA

+55 GGCTTAGTCTGT^GCGGCTGCGGGTCTCT^{-2R}GACTGCCCTGTGGGGAGGGTCTT

+105 GCCTTAACATCCCTTGCAATTTGGCTGCAAAGAAATCTGCTTGAAGAAGG

+155 GGTTACGCTGTTTGGCCGGtgagttttattggcaaaactatcctctggg

+205 tgatgtgtg^{-3R}cctatgctttacaagaattgcctaatttccccaccctgca

HNPP patients. Fifteen CMT1 patients were selected based on a clinical phenotype compatible with CMT1 and severely reduced NCVs. Six cases were familial, of which one case was dominant and one was autosomal dominant, while seven were isolated cases and in two cases there was no family history available. Sixteen unrelated patients were referred to our laboratory as HNPP cases. Two unrelated cases had a positive family history while in 14 HNPP cases there was no information regarding family history. The absence of the CMT1A duplication/HNPP deletion in the patients was confirmed by Southern blot hybridisation of *MspI* digested DNA with markers pVAW409R3a (D17S122) and pEW401HE (D17S61),^{7,8} by short tandem repeat analysis of RM11-GT (D17S122)⁹ and Mfd41 (D17S261),¹⁰ or by pulsed field gel electrophoresis of *FspI* digested DNA hybridised with pVAW409R3a.¹¹ Single strand conformation polymorphism (SSCP) analysis was used to exclude the presence of mutations in the coding regions of PMP22, MPZ, and Cx32¹²⁻¹⁴ in the non-duplicated CMT1 patients and in the coding regions of PMP22 in the non-deleted HNPP patients.

PMP22 expression is regulated by two alternative promoters.¹⁵ The expression of the alternative PMP22 transcripts is tissue specific with high levels of the exon 1A containing transcripts tightly coupled to myelin formation and exon 1B containing transcripts predominating in non-neural tissues and in growth arrested primary fibroblasts.

Three overlapping primer sets were designed for amplification of the promoter 1/exon 1A region. The primer sequences are shown in table 1 and the location of the primers is

Figure 1 Position of the primers for SSCP analysis and sequencing of the non-coding exon 1A (position +1 to +173) and its 5' flanking promoter region. The transcription initiation site of exon 1A is indicated by an asterisk.¹⁵ The C to G transversion at position +67 is shown in bold.

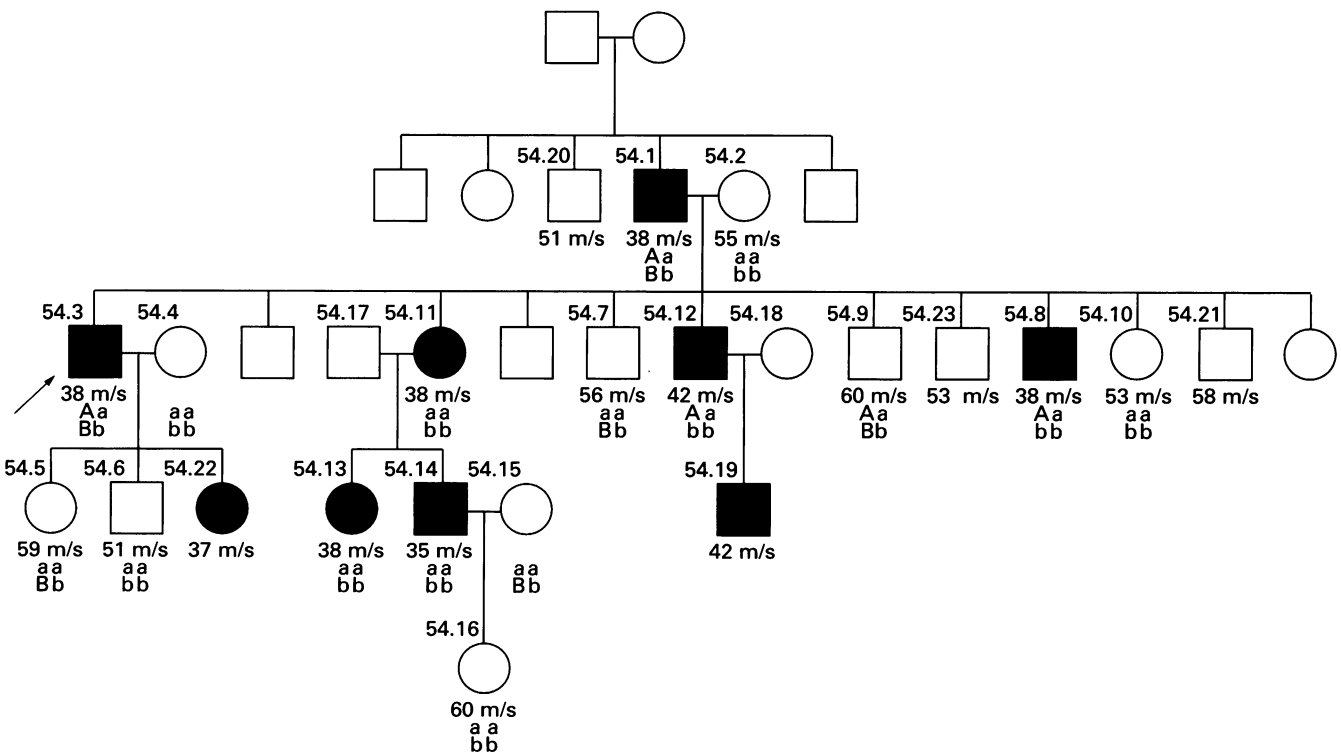


Figure 2 Pedigree of family CMT54. Filled symbols: affected subjects; open symbols: unaffected subjects; arrow: index patient; subjects 54.1-54.16: DNA available for analysis. NCV values of motor median nerve are given below pedigree symbols (mean NCV of patients: 38.4 (SD 4.5) m/s). Segregation of the PMP22 promoter variation (A,a) and the MPZ exon 5 polymorphism (B,b) is shown.

depicted in fig 1. Amplified DNA samples (5 μ l) were mixed with 3 μ l formamide loading dye and, after a two minute denaturation and rapid cooling on ice, subjected to electrophoresis on 1 \times MDE (FMC Bioproducts, Rockland, ME, USA) gels with and without 10% glycerol. Silver staining was carried out as previously described.¹⁴ Although SSCP does not detect 100% of the mutations, the sensitivity is high, particularly when performed under different conditions.¹⁶ In one autosomal dominant CMT1 patient, CMT54.3 (fig 2), a band with altered mobility was detected in fragment 3 comprising exon 1A of the PMP22 promoter region (fig 1). Automated sequence analysis of PCR fragment 3 was performed with the ABI PRISM Dye Terminator Cycle Sequencing kit with AmpliTaq, FS (Applied Biosystems, Foster City, CA) using the same primers as used in the SSCP analysis. Gel electrophoresis and analysis were performed on a 377 DNA sequencer (Applied Biosystems, Foster City, CA). Sequence analysis showed a C to G transversion at position +67 of the 5' non-coding exon 1A of PMP22 (fig 1). CMT54.3 had electrophysiological and electromyographical examination at the age of 41 which showed uniformly slowed motor and sensory NCVs (motor NCV: median nerve 34 m/s, ulnar nerve 34 m/s, and peroneal nerve 27 m/s; sensory NCV: median nerve 36 m/s, ulnar nerve 35 m/s, sural nerve 28 m/s). Neurological examination showed bilateral pes cavus, slight weakness of the peroneal muscles, and distal areflexia in the lower limbs.

To test whether the mutation cosegregated with the disease in family CMT54, we analysed all available family members (fig 2). SSCP analysis indicated that the same mutation was present in the father and in two affected sibs. However, the mutation was also present in one unaffected sib and absent in one affected sib, indicating that the mutation does not segregate with the disease in this family, excluding CMT1A.

Several studies have shown that PMP22 is a dosage sensitive gene. In the majority of the CMT1A cases the disease is caused by overexpression of PMP22 owing to the duplication at 17p11.2. HNPP results from underexpression of PMP22 either by the deletion of PMP22 or a loss of function mutation in the PMP22 coding region. Another possibility is that a mutation in the nerve specific PMP22 promoter could mimic the dosage effect by altered expression of the PMP22 gene. A mutation could increase or decrease the effectiveness of transcription or influence mRNA translation or mRNA stability. Further, a mutation could cause incorrect splicing of the promoter exons or an incorrect translation initiation site could be used. In the case of a mutation in the nerve specific PMP22 promoter, expression in other tissues would not be affected.

The nerve specific promoter of PMP22 was analysed in 15 unrelated CMT1 and 16 unrelated HNPP patients. In one CMT1 patient a band of altered mobility on SSCP analysis was observed and a base variation was identified by sequencing. However, SSCP analysis of all

available family members showed that the mutation did not segregate with the disease, excluding this family as a CMT1A family. Therefore the base change in the non-coding exon 1A of PMP22 is unlikely to be the cause of CMT1 in this family. Previously, SSCP analysis of the coding region of MPZ in proband CMT54.3 had shown a known polymorphism in exon 5.¹⁶ Inspection of the segregation pattern of the MPZ alleles (fig 2) excluded linkage of the disease to the CMT1B locus.

Furthermore, a digenic inheritance model where mutations in two unlinked genes must be present to develop the disease is excluded for PMP22 and MPZ since the affected father, CMT54.1, has transmitted three out of the four possible allelic combinations of both the PMP22 and MPZ polymorphism to his affected children (fig 2). These data suggest that family CMT54 is a non-1A, non-1B, autosomal dominant CMT1 family and confirms that a third autosomal dominant CMT1 locus must exist elsewhere in the human genome.

Our data from the mutation analysis of the PMP22 promoter 1/exon 1A indicate that mutations in the nerve specific PMP22 promoter and untranslated exon will not be a common genetic cause of CMT1A and HNPP. However, we cannot exclude the possibility that a mutation has not been detected by the SSCP method, or that a mutation further upstream of exon 1A may be involved in altering the expression of PMP22.

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