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A Unique Point Mutation in the *PMP22* Gene Is Associated with Charcot-Marie-Tooth Disease and Deafness

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Summary

Charcot-Marie-Tooth disease (CMT) with deafness is clinically distinct among the genetically heterogeneous group of CMT disorders. Molecular studies in a large family with autosomal dominant CMT and deafness have not been reported. The present molecular study involves a family with progressive features of CMT and deafness, originally reported by Kousseff et al. Genetic analysis of 70 individuals (31 affected, 28 unaffected, and 11 spouses) revealed linkage to markers on chromosome 17p11.2-p12, with a maximum LOD score of 9.01 for marker D17S1357 at a recombination fraction of .03. Haplotype analysis placed the CMT-deafness locus between markers D17S839 and D17S122, a ~0.6-Mb interval. This critical region lies within the CMT type 1A duplication region and excludes *MYO15*, a gene coding an unconventional myosin that causes a form of autosomal recessive deafness called DFNB3. Affected individuals from this family do not have the common 1.5-Mb duplication of CMT type 1A. Direct sequencing of the candidate peripheral myelin protein 22 (*PMP22*) gene detected a unique G→C transversion in the heterozygous state in all affected individuals, at position 248 in coding exon 3, predicted to result in an Ala67Pro substitution in the second transmembrane domain of *PMP22*.

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Introduction

Charcot-Marie-Tooth disease (CMT; MIM 118300) (Charcot and Marie 1886; Tooth 1886) is comprised of a clinically and genetically heterogeneous group of polyneuropathies, with a prevalence of ~1 in 2,500 (Skre 1974). It is characterized by degeneration of peripheral nerves, resulting in distal muscle atrophy, sensory loss, and deformities of the hands and feet. Two major dominant clinical types have been defined on the basis of electrophysiological studies (Lupski et al. 1991; Dyck et al. 1992). CMT type 1 is characterized by uniformly decreased nerve conduction velocities (<38 m/s) associated with nerve demyelination. CMT type 2 displays normal to near-normal nerve conduction velocities (>38 m/s), associated with a neuronal defect. The two autosomal dominant types of CMT type 1 are CMT type 1A and CMT type 1B. CMT type 1A (MIM 118220) (McKusick 1992), the most common, accounts for >70% of CMT cases. A 1.5-Mb DNA duplication of chromosome 17p11.2-p12 is observed in most patients with CMT type 1A. A gene-dosage effect resulting in abnormal expression of the *PMP22* gene, which encodes a myelin protein of the peripheral nerves, has been implicated in this disorder (Lupski 1992). In support, point mutations in the *PMP22* gene have been observed in patients with CMT type 1A who do not have the 1.5-Mb duplication (Roa et al. 1993*c;* Navon et al. 1996; Nelis and van Broeckhoven 1996; Marrosu et al. 1997). CMT type 1B (MIM 118200) is associated with point mutations in the myelin protein zero gene (*MPZ, P0*) on chromosome 1q.

CMT with deafness is unusual and has been reported in autosomal dominant (MIM 118300) families. In the family reported by De Weerdt and Heerspink (1974), hearing loss began in individuals at age ~30–40 years and was slowly progressive. Boltshauser et al. (1989) described hereditary motor-sensory neuropathy (HMSN), vocal cord paralysis, and progressive sensorineural hearing loss in three individuals in a three-gen-

eration family. One study (Gadoth et al. 1991), involving 16 patients from five families, documented autosomal dominant HMSN type I in three isolated individuals with a history of hearing impairment and showed sensorineural hearing loss in five of seven patients who underwent audiometry.

Linkage studies were performed with one family from Israel exhibiting CMT and sensorineural deafness, features of which were apparent in early childhood and infancy (Hamiel et al. 1993). Duplication of the 17p11.2-p12 locus commonly associated with CMT type 1A was not observed, and linkage to this locus was excluded by the use of molecular markers mapped within this region. CMT type 1B was also excluded through phenotyping of the Duffy blood group linked to chromosome 1q21-q23. Molecular studies were not performed in earlier reports of autosomal dominant deafness and motor-sensory neuropathy. An X-linked motor-sensory neuropathy with deafness and mental retardation (MIM 310490) was found to link to Xq24-26 (Cowchock et al. 1985; Priest et al. 1995). Autosomal recessive CMT and deafness (MIM 214370) was also reported in three sons of first-cousin parents (Cornell et al. 1984). Kalaydjieva et al. (1996) reported linkage to chromosome 8q24 in a Gypsy community from Bulgaria with autosomal recessive HMSN, deafness, and unusual neuropathological features (MIM 601455).

Rapid progress has been made in the identification of genes associated with deafness. To date, ⩾20 autosomal dominant and 25 autosomal recessive forms of nonsyndromic deafness have been mapped, and 15 of the relevant genes have been cloned (Hereditary Hearing Loss home page). Two autosomal dominant deafness loci colocalize with CMT. CMT type 1B and deafness locus DFNA7 (Fagerheim et al. 1996) both map to 1q21-q23. DFNA12 (Verhoeven et al. 1997; Kirschofer et al. 1998) colocalizes with CMT type 4B on chromosome 11q22-q24. The corresponding deafness genes for these loci are unknown. The deafness locus DFNB3 was mapped to chromosome 17p11.2-p12 in families from Bengkala, Bali, with autosomal recessive deafness (Friedman et al. 1995). Subsequently, mutations in the myosin gene *MYO15* have been observed in affected individuals in three unrelated families in Bali (Wang et al. 1998).

Our study involves a large central Illinois family with autosomal dominant CMT and deafness, originally reported by Kousseff et al. (1982). Most early reports did not evaluate CMT in association with hearing loss molecularly or cytogenetically. The single linkage study of autosomal dominant CMT and deafness excluded both the CMT type 1A and type 1B loci. Because of these earlier studies, our hypothesis was that a unique gene was responsible for deafness with the CMT phenotype.

Subjects, Material, and Methods

Ascertainment of Family Members

Members of a seven-generation central Illinois family, segregating for CMT with deafness, were included in the present study (fig. 1). Informed consent was provided by all participants prior to their inclusion in the study. Individuals received a neurological examination in addition to comprehensive audiological and nerve-conduction studies to establish affection status. Affected individuals have degeneration of peripheral nerves resulting in distal muscle atrophy, sensory loss, loss of reflexes, and deformities of hands and feet. Most affected adult individuals report sensorineural deafness.

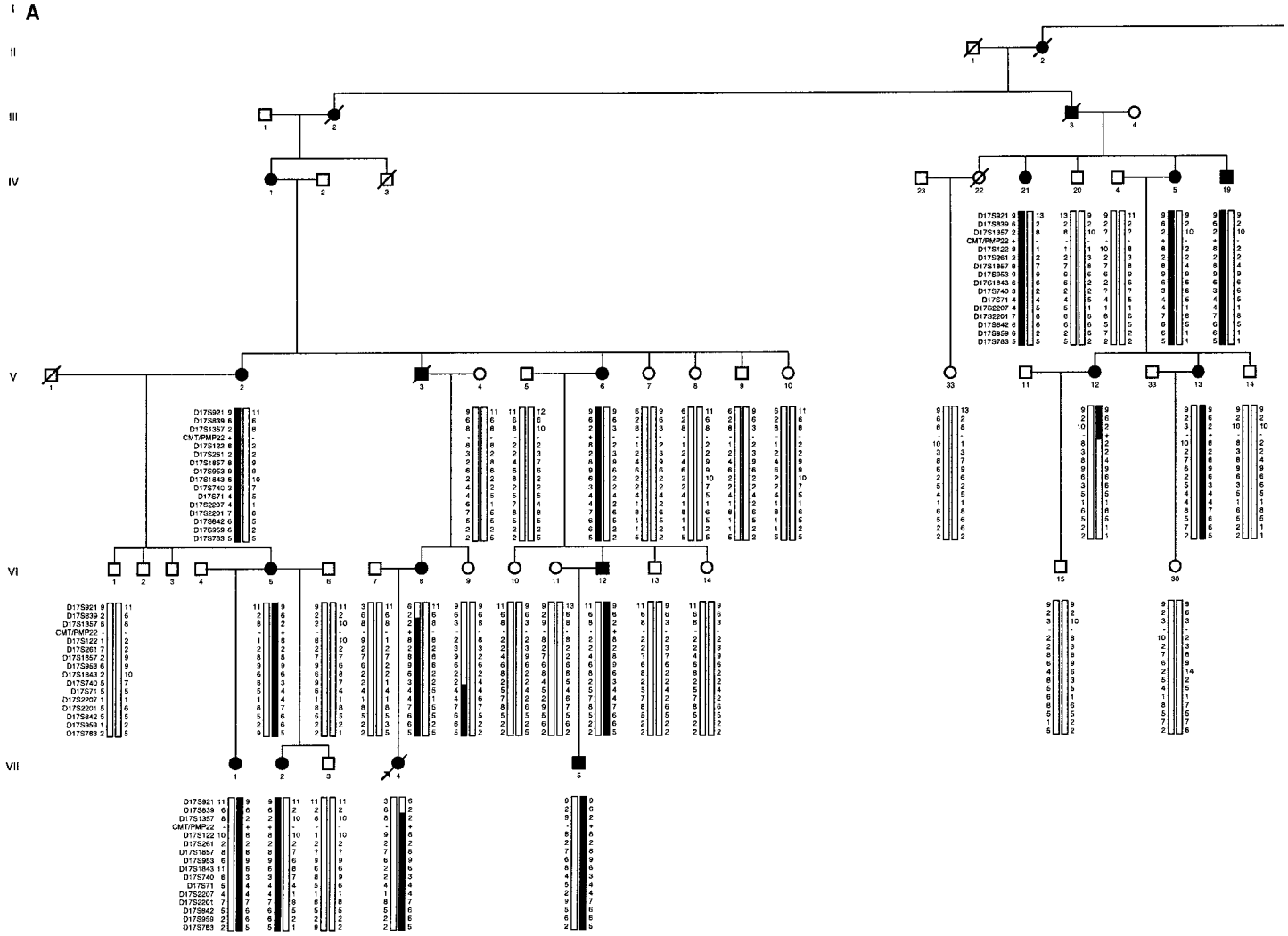
To evaluate for the CMT type 1A duplication, cytogenetic and molecular analyses were performed by commercial laboratories. Athena Diagnostics performed restriction endonuclease digestion and Southern blot analysis of pulse-field electrophoresed genomic DNA from one affected individual (pedigree identity V:30). *Sac*II-digested DNA was hybridized with a CMT type 1A repeat-element probe to visualize disease-specific junction fragments (Lorenzetti et al. 1995). FISH analysis of interphase nuclei with cosmid contigs spanning the *PMP22* locus was also performed on affected individual V:30 (Kleberg Cytogenetics Laboratory; Shaffer et al. 1997).

Audiological Assessment

Family members had a comprehensive audiological test battery, including pure-tone air and bone conduction thresholds, speech reception thresholds, word recognition, tympanometry, acoustic reflex measures, auditory brain stem response testing, middle latency responses, spontaneous otoacoustic emissions, transient otoacoustic emissions, and distortion product otoacoustic emissions.

DNA-Marker Analysis

Peripheral venous blood was obtained by standard venipuncture. Genomic DNA was extracted by means of the Genomix DNA extraction kit (TEL-TEST). In cases where blood samples could not be obtained, buccal scrapings were collected and processed with standard methods. An initial genome scan was performed with microsatellite markers (Research Genetics) to exclude or include linkage to known chromosome locations of several types of CMT: D17S1843 (CMT type 1A, 17p11.2-p12), D1S2635 and CRP (CMT type 1B, 1q21-q23), D12S342 (CMT type 2, 12q24), D1S199 and D1S228 (CMT type 2A, 1p35-36), D3S1551 (CMT type 2B, 3q13-q20), D8S551 (CMT type 4A, 8q13-q21.1), and D11S900 (CMT type 4B, 11q23). A maximum LOD


A
I
II
III
IV
V
VI
VII
D17S921
D17S839
D17S1357
CMT/PMP22
D17S122
D17S261
D17S1857
D17S953
D17S1843
D17S740
D17S71
D17S2207
D17S2201
D17S842
D17S959
D17S783

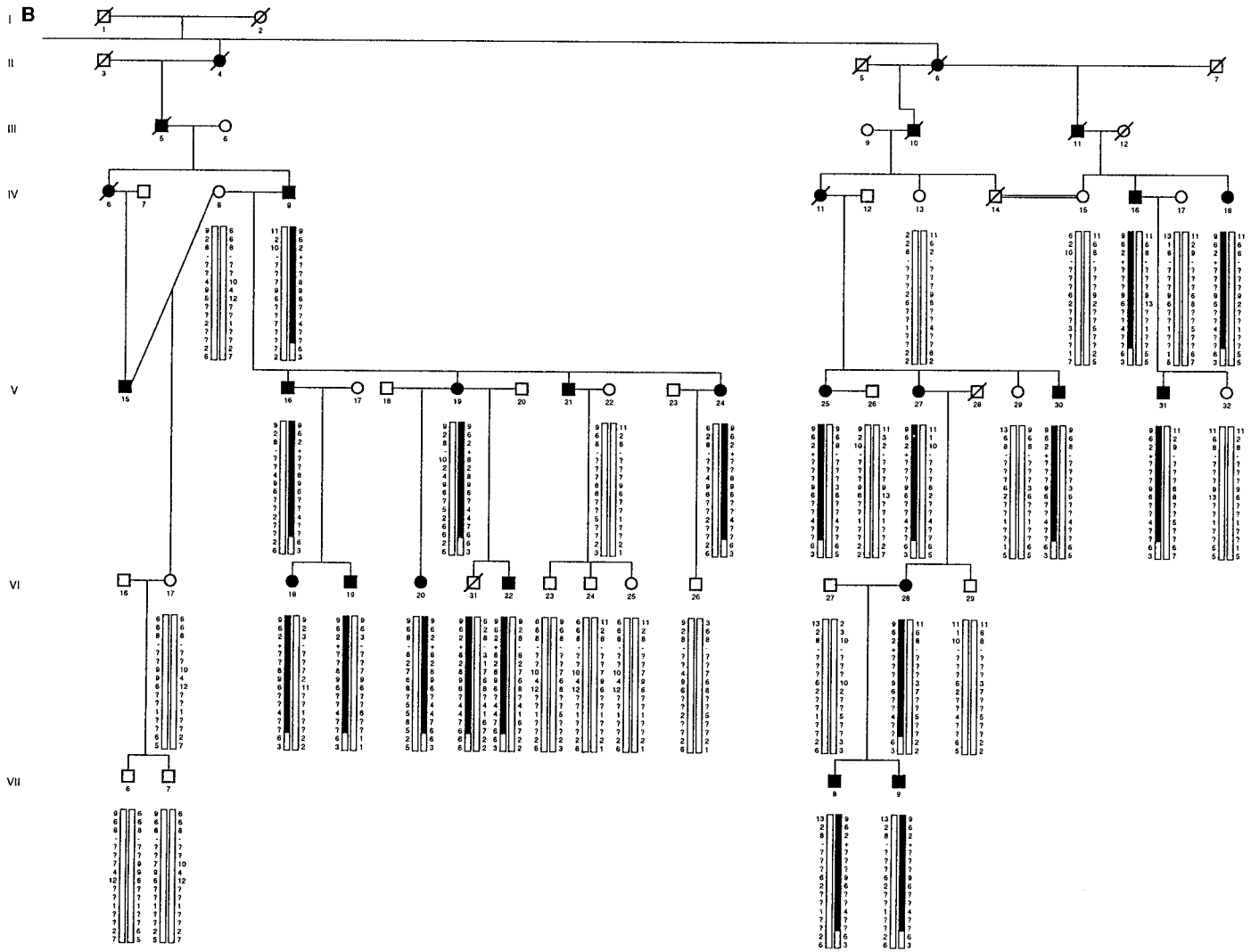


Figure 1 Pedigree of family affected with autosomal dominant CMT and deafness showing haplotype analysis for 15 markers on 17p11.2. Markers are listed top to bottom: telomere-D1S921-D17S839-D17S1357-CMT-D17S122-D17S261-D17S1857-D17S953-D17S1843-D17S740-D17S71-D17S2207-D17S2201-D17S842-D17S959-D17S783-centromere. For each marker, the alleles were numbered on the basis of sizes reported in the Genome Database. Novel alleles were assigned numbers after the last reported allele in the Genome Database. The site of the *CMT1A/PMP22* gene locus is included in relation to its position to the molecular markers. A plus sign (+) designates the disease allele; a minus sign (−), the nondisease allele. The boldfaced bars represent the disease haplotype linked to CMT with deafness. Circles denote females, squares denote males, slashed symbol denotes deceased individuals, blackened symbol denotes affected individuals. An arrow indicates the proband.

score of 6.31, with marker D17S1843 at recombination fraction (θ) = .03, indicated linkage to chromosome 17p11.2-p12. Linkage refinement was conducted with additional chromosome 17 markers: D17S921, D17S839, D17S1357, D17S122, D17S261, D17S1857, D17S953, D17S1843, D17S740, D17S71, D17S2201, D17S842, D17S959, and D17S783. Marker D17S2207, a highly polymorphic $(CA)_n$ microsatellite repeat located upstream of the *DFNB3/MYO15* gene, was used to analyze linkage to this deafness locus (kindly provided by Dr. Thomas B. Friedman, National Institute on Deafness and Other Communication Disorders [NIDCD]). Amplification was performed with forward primers tagged with fluorescent dyes, as provided by manufacturer. Primers not provided in the tagged form were end-labeled with fluoroscein with the 5′-oligolabelling for fluorescence kit (Amersham Pharmacia Biotech). Amplification products were analyzed on a 6% denaturing polyacrylamide gel and visualized with an FMBIO-100 fluorescent image scanning unit (Hitachi). A PowerPlex allelic ladder (Promega) was loaded onto each gel to permit sizing of individual alleles. Alleles were scored, and genotype data were entered into the pedigree file of the LINKAGE computer package.

Linkage Analysis

A standard LOD-score approach for linkage analysis was used with the software LINKAGE, for the two-point analyses, and with the VITESSE program, for multipoint analysis (O'Connell and Weeks 1995). To allow for children who have not yet shown any clinical evidence of CMT, age-dependent penetrance was taken into account in the genetic model of autosomal dominant disease. The four dependent risk classes identified were I: 0–6 years old, .176; II: 7–20 years old, .588; III: 21–31 years old, .893; and IV: ⩾32 years, .999. The genetic maps of the markers and allele frequencies were taken from the Genome Database. When allele frequency information was unavailable, the allele frequency was obtained from pedigree data.

Mutation Analysis of PMP22

We amplified the four coding exons of *PMP22* from genomic DNA of affected and unaffected individuals, using exon-specific primer pairs (Roa et al. 1993*c*). The two noncoding exons were amplified with designed primers based on the published sequences (Suter et al. 1994). These primers were as follows: exon 1A forward primer sequence: 5′-CCAGTGGGACCTCTTGGCTA-3′; exon 1A reverse primer: 5′-GCACAGTTTGCCAA-TAAAAC-3′; exon 1B forward primer: 5′-GGCATCCG-CTGAGCTACATT-3′; and exon 1B reverse primer: 5′-CCATCTCCTTCACTCTCCCC-3′. PCR reactions consisted of 200 ng genomic DNA and 0.8 μM appropriate primers. PCR conditions were as described by Roa et al. (1993*c*). All amplifications were done at an annealing temperature of 60°C, except amplification of exon 2, in which an annealing temperature of 55°C was used. The PCR products were purified from a 3% agarose gel (NuSieve 3:1; FMC Bioproducts) prior to analysis.

For sequence analysis, 1/20th of the initial PCR sample (gel-purified) was subjected to a second-round PCR as described, for 25 cycles. These products were purified from agarose, and ~100 ng were used in cycle sequencing reactions according to manufacturer's protocol (Big Dye Terminator Cycle Sequencing; PE Applied Biosystems). Both sense and antisense strands were sequenced. After thermocycling, the reactions were precipitated with ethanol, lyophilized, and sent off to a sequencing facility for heterozygosity analysis (ACGT).

For *Hae*III restriction-fragment–length polymorphism (RFLP) analysis, exon 4 (coding exon 3) was amplified from genomic DNA of family members and spouses, as well as from 52 unrelated individuals. Each PCR reaction was precipitated with ethanol in the presence of yeast tRNA (0.5 μg) as carrier nucleic acid. The samples were washed with 70% ethanol, lyophilized, and resuspended in 5 μl deionized water. Each sample was digested for 16 h with 5 U *Hae*III (New England BioLabs) in a 10-μl final volume of 1 × reaction buffer, supplied by the manufacturer. The complete reactions were analyzed on a 3% NuSieve 3:1 (FMC BioProducts) agarose gel containing 0.25 μg/ml ethidium bromide.

Results

Clinical, Electrophysiological, and Pathological Studies

Members of the family studied included 31 affected individuals, 28 unaffected individuals, and 11 spouses. The proband (VII:4) for this study was given a diagnosis at birth of bilateral vocal cord palsy, swallowing incoordination, and hypotonia. The respiratory distress was managed with tracheostomy at birth, and the swallowing incoordination was managed by gastrostomy tube placement at age 3 wk. She had decreased tendon reflexes. Her brain stem auditory-evoked responses were abnormal, although otoacoustic responses were normal, indicating normal outer hair cells function. Nerve-conduction studies indicated slow conduction velocities. At age 18 mo she died from respiratory compromise. Her mother (VI:8) reported having had, in her teens, muscle weakness that caused difficulty in walking, husky soft voice, and hearing loss, and her maternal grandfather had developed mild features of CMT in his twenties. In addition to the proband, another male infant (VI:31) died at age 3 mo, from weakness, hypotonia, and feeding difficulties. A diagnosis was not established at autopsy to explain his death. Linkage and mutation analysis,

with autopsy material from histological analysis, indicated that he had inherited his mother's disease allele.

From the clinical data accumulated, increasing clinical severity and younger age at onset of the neurological features and deafness with each progressive generation was indicated, which is indicative of anticipation. The mean age at onset of symptoms of CMT in different generations was 12, 9.3, 3.8, and 4.4 years for generations IV, V, VI, and VII, respectively (fig. 1). Although the difference was not statistically significant for the entire family ($P = .09$), when each generation was analyzed separately for each individual branch of the family, there was evidence of earlier age at onset ($P = .009$). The mean ages reported for onset of deafness in the three generations—generations IV, V, and VI—were 41, 18.4, and 12.9 years, respectively. The age difference in each generation was statistically significant ($P = .004$). Deafness and features of CMT are progressive in this family. Because many individuals in the youngest generation do not have documented deafness and are too young to cooperate with the comprehensive audiology research studies, generation VII was not included in the latter analysis.

All affected individuals have had routine audiology studies. Nineteen affected and eight unaffected family members have had comprehensive audiological assessments. The details are described in a separate manuscript (K. Campbell, L. Hughes, L. Mazzeo, M. Kovach, V. Kimonis, unpublished data). The sensorineural hearing losses in the affected family members ranged from borderline normal to profound hearing loss, with all having at least mild bilateral hearing loss by adulthood. Word recognition was often poorer than would be predicted from pure-tone thresholds, which is consistent with auditory neuropathy.

A variety of abnormalities consistent with peripheral auditory neuropathy were noted on the auditory electrophysiological test battery for all affected family members. More than half the affected family members had a clearly replicable cochlear microphonic, with almost half of those showing a marked enlargement. The cochlear microphonic, which emanates from the AC electric activity of the cochlear outer hair cells, has been reported to be markedly enlarged in some cases of auditory neuropathy, and this enlargement may be pathognomonic to that disorder.

The auditory brain stem response (ABR), measuring auditory electric responses from the VIIIth nerve through the lateral lemniscus, was abnormal in all affected patients, which is consistent with otoneurological abnormality. The most common abnormality was delayed waveform latencies. In the remainder of the affected patients, all ABR waveforms were absent. Middle latency responses, measured through the auditory thalamocortical area, were less commonly affected but still were either absent or of poor morphology in >50% of the affected patients.

Acoustic reflex abnormalities were also present. These abnormalities included elevated acoustic reflex thresholds, absent acoustic reflexes, or acoustic reflex decay. Acoustic reflex studies were normal in only one affected patient.

The results of the otoacoustic emissions testing, sensitive measures specifically of cochlear function, also yielded consistently abnormal results, clearly suggesting cochlear involvement as well. Audiological studies of patients with CMT disease and deafness have suggested that the hearing impairment may be entirely neural (Gadoth et al. 1991), but in the family reported in the present study, a concomitant cochlear component is present. The patients report that their hearing losses are progressive, but longitudinal studies have not yet been performed.

Nerve-conduction velocities are slow (mean motor and sensory 24 m/s), suggestive of CMT type 1A. Electron microscopy of a sural nerve obtained from the mother of the proband revealed "onion bulb" whorls composed of multiple Schwann-cell lamellae surrounding demyelinated or thinly myelinated axons. Results of electron microscopy of muscle were normal.

Molecular and Cytogenetic Analysis for CMT Type 1A Duplication

Pulsed-field gel electrophoresis and Southern blot analysis performed on affected individual V:30 did not detect the 500-kb junction fragment diagnostic of the CMT type 1A common duplication. The absence of the duplication at 17p11.2-12 was confirmed by FISH analysis of interphase nuclei from the same individual and by PCR assay with polymorphic microsatellite marker D17S1357 (Blair et al. 1995).

Linkage Analysis

CMT types 1B, 2, 2A, 2B, 4A, and 4B were excluded by LOD scores of $\leqslant -2.00$. A maximum LOD score (Z_{max}) of 6.31 at $\theta = .10$ for marker D17S1843 indicated linkage to the CMT type 1A locus on chromosome 17p11.2-p12. To ascertain whether there was cosegregation with the deafness locus DFNB3, a high-density map of this region was obtained by genotyping of individuals with additional closely spaced markers that spanned the genetic interval between D17S921 and D17S783. A Z_{max} of 9.01 was obtained for D17S1357 at $\theta = .03$. Multipoint LOD score analysis gave a Z_{max} of 11.1.

Haplotype Analysis

To determine whether the smallest interval containing the CMT with deafness locus included the *MYO15* gene,

we analyzed individuals for recombination events by using haplotype reconstruction (fig. 1). The cosegregating segment in which no recombination was detected was flanked by the markers D17S839 and D17S122.

Figure 2 summarizes the conclusions derived from haplotype analysis. The critical region defined by markers D17S839 and D17S122 spans ~0.6 Mb of 17p11.2 (a detailed map of 17p11.2-p12 duplication region was kindly provided by Dr. J. R. Lupski). The only marker for which recombinations have not been observed is D17S1357. This marker is within the same bin as *PMP22* and has been used to detect the CMT type 1A duplication (Blair et al. 1995), highly suggestive of *PMP22,* representing the candidate gene for this disorder. The *MYO15* gene has been excluded as a result of recombinations observed.

Mutation Analysis

Mutation analysis was performed to determine whether affected individuals in this family carried mutations in *PMP22,* because CMT with deafness maps to chromosome 17p11.2-p12, the CMT type 1A locus, and affected individuals do not carry the common 1.5-Mb duplication associated with 70% of CMT type 1A cases. Heteroduplex analysis (Roa et al. 1993*c*) did not detect mutations in the *PMP22* gene. Sequence analysis of all coding and noncoding exons was also performed on one unaffected individual and three affected individuals. Both strands of the generated PCR products were sequenced. A G→C transversion was detected at position 248 in exon 4 (coding exon 3) in the heterozygous state (fig. 3*A*). This mutation is predicted to result in a single amino acid substitution of proline for alanine at codon 67. Mutations were not detected in any other exon.

The G→C point mutation results in the loss of a cleavage site for restriction enzymes *Hae*III and *Bst*NI. All individuals in the pedigree were subjected to RFLP analysis of exon 4 with *Hae*III (fig. 3*B*). Digestion with *Hae*III produced two fragments of 159 and 55 bp in all unaffected individuals. Individual VI:9 is only 8 years old, however, and thus far exhibits no clinical features of CMT. She shares the disease haplotype for markers D17S71, D17S2207, D17S2201, D17S842, D17S959, and D17S783 but did not inherit the critical region of linkage. RFLP analysis with *Hae*III indicated that she was unaffected. The RFLP pattern in affected individuals reflected the heterozygous state, generating three products 214, 159, and 55 bp in length. This mutation was not observed in spouses of family members or in 52 unrelated controls.

Discussion

CMT is rarely associated with deafness but is observed sporadically in patients with peripheral neuropathies. A large family exhibiting autosomal dominant CMT and deafness provided a unique opportunity for molecular analysis of this distinct disorder. Linkage studies of CMT and deafness in the family reported by Hamiel et al. (1993) excluded CMT types 1A and 1B loci. Our findings indicate linkage to CMT type 1A chromosomal locus 17p11.2-p12. Two candidate genes, *MYO15* of the DFNB3 locus and *PMP22* of the CMT type 1A locus, map to this region. Homozygous mutation in *MYO15,* which encodes an unconventional myosin, leads to autosomal recessive nonsyndromic deafness (Friedman et al. 1995; Wang et al. 1998). Because dominant and recessive forms of deafness can be caused by genetically distinct mutations within the same gene (Tamagawa et al. 1996; Weil et al. 1997), *MYO15* was evaluated as a possible candidate gene with high-density marker and haplotype analysis. Recombinations with the disease locus and genetic markers telomeric of D17S122, however, excluded the DFNB3 locus (fig. 1 and 2).

A search of the Human Cochlear EST Database reveals that *PMP22* is presently the only known gene expressed in the cochlea that maps to position 17p11.2-p12. It is a 160–amino acid–membrane glycoprotein highly expressed by Schwann cells in compact myelin of the peripheral nervous system (Snipes et al. 1992). Duplication of *PMP22* and, occasionally, point mutations

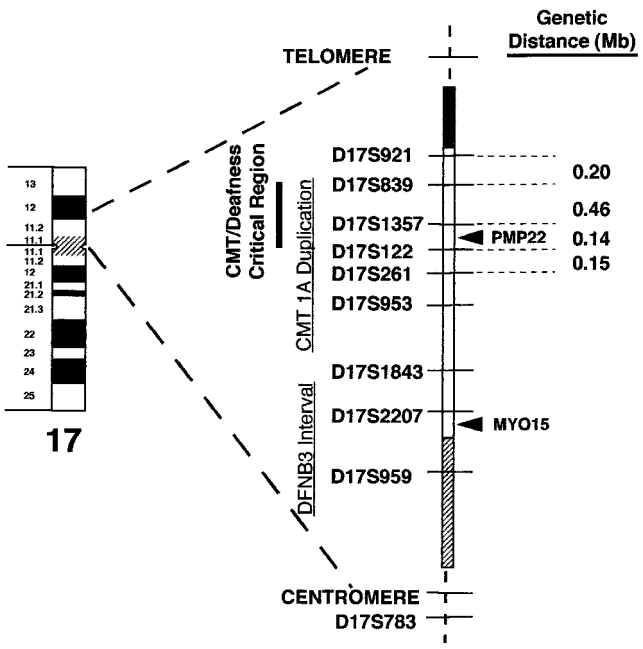


Figure 2 Genetic map of chromosome 17p11.2 markers. The sites for the autosomal recessive deafness (*DFNB3*) locus and CMT type 1A duplication region are indicated. The CMT and deafness critical region, defined by markers D17S839 and D17S122, is represented by a boldfaced bar. Genetic distances and placement of *PMP22* were obtained from PAC/BAC map of the CMT type 1A duplication region (personal communication, Dr. J. R. Lupski).

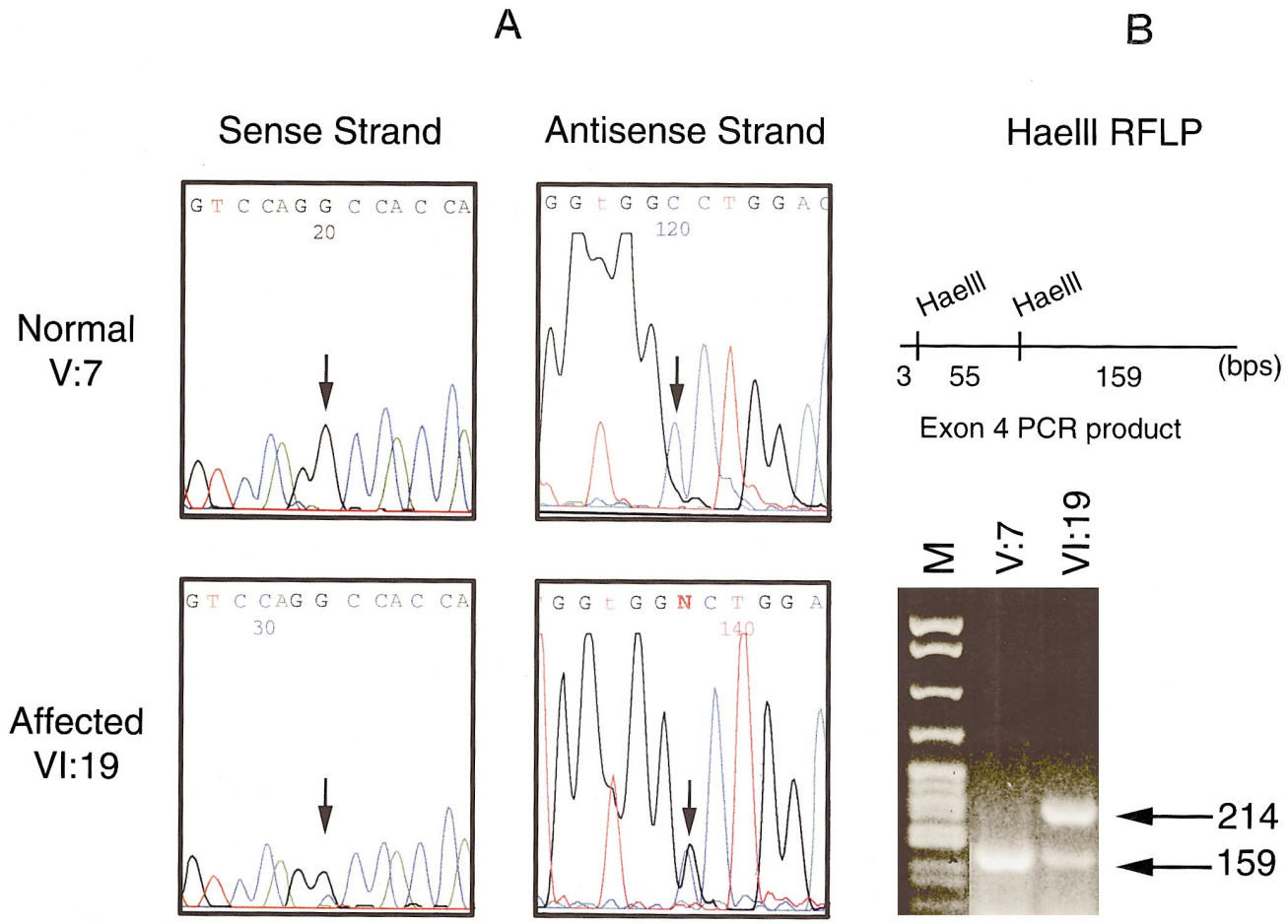


Figure 3 *A*, Identification of the G→C mutation in *PMP22* exon 4. The sense and antisense sequences are shown for an unaffected (V:7) and an affected (VI:19) family member. An arrow indicates the location of the G→C heterozygous point mutation. Note that the automatic sequence generated miscalled the mutated nucleotide in the sense strand, but the peak height has changed in the affected individual. *B*, Confirmation of G→C heterozygous point mutation by RFLP analysis. This point mutation results in the loss of a restriction cut site for enzyme *Hae*III. Digestion of genomic DNA from the unaffected individual (V:7) produced two fragments of 159 and 55 bp. RFLP analysis of the affected individual (VI:19) generated three fragments of 214, 159, and 55 bp, representing the heterozygous state of mutation. The 55-bp restriction fragment is not shown in this gel.

within the gene have been implicated in the cause of CMT type 1A. It has been proposed that abnormal expression (a gene-dosage effect due to the duplication) and altered protein structure of PMP22 results in impaired function of peripheral myelin in patients with CMT (Lupski 1992; Snipes et al. 1992). Sequence analysis of *PMP22* revealed a unique dominant missense mutation that cosegregates with deafness and CMT in the family studied.

Table 1 presents known mutations in *PMP22*. Including the mutation in this report, ⩾24 autosomal dominant mutations are known. Of the 19 missense mutations, 14 are the result of nonconservative substitutions. With the exception of Gly93Arg, which is located in the intracellular loop between transmembrane domain 2 (TM2) and TM3 (fig. 4), all the amino acid changes occur within the transmembrane domains. Point mutations in *PMP22* are commonly associated with Dejerine-Sottas syndrome (DSS; MIM 145900), a clinically more severe neuropathy than CMT. Those affected with DSS typically have nerve-conduction velocities <10 m/s and have an earlier age at onset than that of patients with CMT type 1A duplication (Roa et al. 1993*a*; Valentijn et al. 1995). Classic CMT has also been reported to be caused by point mutations in *PMP22*. There does not appear to be a defined category of *PMP22* point mutations that can distinguish between CMT and DSS; therefore, DSS and CMT represent a spectrum of related disorders.

Mutations responsible for the clinically reported

Table 1

***PMP22* Point Mutations**

Clinical Description and Mutation	Residue	Reference
DSS:		
T→A transversion	Met69Lys	Roa et al. 1993*a*
G→T transversion	Gly150Cys	Ikegami et al. 1998
G→A transition	Gly150Asp	Ionasescu et al. 1997
G→A transition	Gly100Glu	Marques et al. 1998
G→A transition	Gly100Arg	Bort et al. 1997
T→C transition	Ser79Pro	Bort et al. 1998
C→A transversion	His12Gln	Valentijn et al. 1995
C→G transversion	Ser72Trp	Tyson et al. 1997
T→C transition	Leu80Pro	Tyson et al. 1997
2-bp insertion	Shift at Leu80	Ikegami et al. 1998
C→T transition	Ser72Leu	Roa et al. 1993*a*
C→T transition	Ser72Leu	Ionasescu et al. 1995
C→T transition	Ser72Leu	Marques et al. 1998
DSS and hearing loss:		
C→T transition	Ser72Leu	Ionasescu et al. 1996
G→T transversion	Ser76Ile	Tyson et al. 1997
CMT type 1A and hearing loss:		
G→C transversion	Ala67Pro	Present study
CMT type 1A:		
C→G transversion	Ser79Cys	Roa et al. 1993*b*
T→G transversion	Leu147Arg	Navon et al. 1996
IVS3+1, splice site	TGgt→TGat	Nelis and van Broekhoven 1996
T→C transition	Leu16Pro	Valentijn et al. 1992
G→T transversion	Gly107Val	Marrosu et al. 1997
G→C transversion	Gly93Arg	Ohnishi et al. 1995
CMT type 1A, recessive:		
C→T transition	Thr118Met	Roa et al. 1993*b*
HNPP:		
2-bp deletion	Stop at Ser41	Nicholson et al. 1994
1-bp deletion	Stop at Val154	Taroni et al. 1995
IVS1+1, splice site	GCgt→GCtt	Bort et al. 1997
1-bp deletion	Shift at Arg95	Young et al. 1997
G→A transition	Val30Met	Sahenk et al. 1998
Trembler mouse mutants:		
Trembler	Gly150Asp	Suter et al. 1992*b*
Trembler-J	Leu16Pro	Suter et al. 1992*a*

CMT/DSS cases are dispersed throughout all four transmembrane domains and one intracellular domain. Individual missense mutations at codon 79 resulted in two distinct clinical phenotypes: Ser79Cys manifested as CMT (Roa et al. 1993*c*) and Ser79Pro manifested as DSS (Bort et al. 1998). The structural/biochemical nature of the substituted residue may influence the phenotype; however, it is not unusual for the CMT phenotype to vary, as observed even among members of the same family who carry identical mutations.

Although PMP22 involvement in deafness has not been addressed, two isolated cases of de novo DSS and hearing loss have been reported (Ionasescu et al. 1996; Tyson et al. 1997). Both of these mutations occur within the second transmembrane domain of PMP22 (fig. 4) and result in polar to nonpolar amino acid substitutions for codons 72 and 76, respectively. Ser72 appears to be a mutational hotspot, with Ser72Leu mutations being reported in three de novo cases of DSS with severe clinical features without deafness (Roa et al. 1993*a;* Ionasescu et al. 1995; Marques et al. 1998) and a single de novo case of DSS associated with deafness (Ionasescu et al. 1996). The latter patient was a significantly developmentally delayed 32-year-old woman who was wheelchair bound. She had facial weakness, nystagmus, and scoliosis, which required surgery. Her nerve-conduction velocity was 11 m/s. The patient was heterozygous for the mutation, suggestive of a dominant allele, and did not carry the common CMT type 1A duplication. None of the other three patients associated with the Ser72Leu mutation had reported deafness. Details of her sensorineural hearing loss, which the authors speculated could have been acquired, were not available from the report. An isolated case reported by Tyson et al. (1997), associated with the Ser76Ile exchange in TM2, was that of a 39-year-old woman whose hearing was impaired for

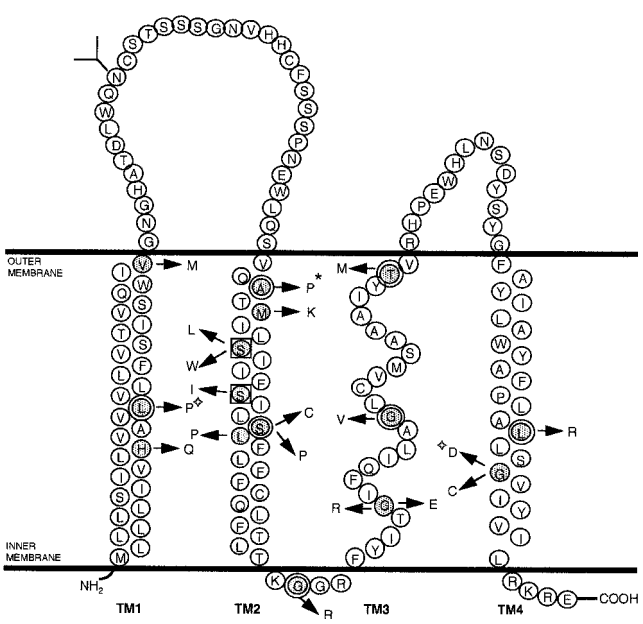


Figure 4 Proposed protein structure of PMP22 (figure adopted from Roa et al. 1993*a*). PMP22 is predicted to have four transmembrane domains (TM1–TM4) and one *N*-glycosylation site (N41) (Suter et al. 1992*a*). A β-sheet conformation is predicted for domains TM1, 2, and 4, whereas TM3 is thought to be α-helical in nature. The 160 amino acids of PMP22 are represented in single-letter code. Substitutions because of single-base point mutations are shaded, with an arrow pointing to the designation of the changed residue. The unique substitution associated with CMT and deafness (Ala67Pro) reported in this paper is represented by an asterisk (*). Point mutations observed in patients given a clinical diagnosis of CMT are double encircled. Circles wrapped by a square designate the two mutations in which deafness was reported elsewhere, in addition to CMT. Mutations identical to the trembler (Gly150Asp) and trembler-J (Leu16Pro) mouse genotypes are indicated by a diamond (◇). Multiple substitutions have been reported for three residues (Ser72, Ser79, and Gly100).

10 years, but details of audiological testing were not available from the report. Deafness was not reported in the other cases associated with mutations in *PMP22*.

We report a unique missense mutation in *PMP22* resulting in an amino acid exchange (Ala67Pro), also in TM2. This mutation is associated with CMT and deafness, an unusual phenotype. Proline is a structurally rigid, rarely used amino acid that causes sharp bends in peptide chains and most likely destabilizes the predicted β-sheet conformation of TM2. It has been speculated that PMP22 acts as a pore or channel protein (Suter et al. 1992*a*) or is involved in interactions between axons and Schwann cells (Snipes et al. 1993). The proximity of the destabilizing proline substitution to the extracellular domain could significantly affect extracellular interactions of PMP22 with itself or other molecules, as well as interfere with proper integration into the myelin membranes.

The basis of hearing loss in this family is intriguing. In light of the high levels of *PMP22* transcript detected in the cochlea (Robertson et al. 1994), a relationship between deafness and the unique *PMP22* mutation observed in this family is an attractive theory. Examination of *PMP22* involvement in myelination and nerve conduction in this tissue should prove interesting. In the auditory system, the VIIIth nerve, surrounded by Schwann cells, would be the most likely site of auditory neuropathy for this family. The prolongation of ABR interpeak latencies and/or absence of ABR waveforms would generally be consistent with this putative site of lesion.

VIIIth-nerve abnormalities should most notably prolong the I–III interpeak interval of the ABR or cause absence of earlier waveforms with only a delayed wave V present. These findings were typical in this family. However, some individuals also showed III–V prolongations, suggesting involvement of the central auditory pathways. Although *PMP22* mutations may primarily affect the periphery, *PMP22* mRNA has been found in the central nervous system, particularly during development (Parmantier et al 1995). Central auditory pathways are myelinated from the brain stem through cortical levels, although *PMP22* expression specifically in those pathways has not yet been investigated.

The mechanisms of cochlear involvement in this family, as reflected in otoacoustic emission abnormalities, are not well defined. Whereas *PMP22* is abundantly expressed by Schwann cells of the peripheral nervous system, comparatively lower levels of *PMP22* transcripts have also been detected in nonneural cell types (Welcher et al. 1991), where their expression is regulated in a growth-specific manner. Therefore, *PMP22* might have a hitherto unknown role in regulation of cell growth and cell-to-cell interactions and, consequently, cochlear development.

Unlike CMT associated with the *PMP22* duplication and a gene-dosage effect, the Ala67Pro mutation is likely to cause a dominant-negative effect, like the majority of DSS and CMT point mutations. Because these other *PMP22* allele variant mutations are not associated with an obvious gross impairment of hearing, the phenotypic differences seen with the Ala67Pro mutation probably indicate either a codon-specific effect or a genetic background effect.

Codon-specific effects are well described for other pore or channel proteins, including several that are important in hearing (Kelsell et al. 1997; Xia et al. 1998). The X-linked form of CMT (CMTX), caused by mutations in connexin 32 (*GJB1*) (Bergoffen et al. 1993), is sometimes coupled with mild deafness (Nicholson and Corbett 1996). Although the pathophysiogical basis for hearing loss in CMTX has not been studied at the molecular level, mutations in two other gap junction proteins, connexin 26 (*GJB2*) (Kelsell et al. 1997) and con-

nexin 31 (*GJB3*) (Xia et al. 1998), are associated with hearing loss. Connexin 26 transcripts have been isolated from the cochlea, and expression of the connexin 31 has been identified in inner ear tissue (Xia et al. 1998). Because *GJB1* can form heterotypic channels with *GJB2* (White et al. 1995), the finding of mild deafness with some forms of CMTX is not surprising. The protein structure of PMP22 bears a close resemblance to connexins, is also expressed cochlearly, and could play a role in cochlear function similar to that speculated for connexins in CMTX with deafness.

Clinical data for this family are suggestive of anticipation. An increased severity and younger age at onset of the neurological features and deafness have been observed with each progressive generation. Two affected individuals aged <2 years died from severe manifestation of CMT. Trinucleotide-repeat expansion is often associated with anticipation in neurodegenerative disorders. The majority of these genetic diseases are characterized by enlargement of the normally polymorphic CAG/CTG or CGG/CCG repeats (Ashley and Warren 1995). Friedreich's ataxia is a trinucleotide-repeat–expansion disorder distinguished by an intronic GAA triplet repeat expansion (Campuzano et al. 1996).

A correlation between disease severity and triplet repeat length was not observed in this family (data not shown). Although generational expansion was not detected in either maternal or paternal modes of transmission, the Repeat Expansion Detection (RED) assay used (Schalling et al. 1993) is a genomewide search of triplet repeat expansions, and small-scale expansions could potentially be masked by the normal level of triplet repeat lengths present within the genome of an individual.

This is the first report of a stable, dominant, unique mutation in *PMP22* causing deafness in addition to CMT. The phenotypic variability and anticipation of CMT in this family are difficult to explain by a single gene mutation, but this has been reported in other disorders (Peral et al. 1996; Fraser 1997). Marrosu et al. (1997) also noted an inconsistent genotype-phenotype correlation and an anticipation-like phenomenon in a five-generation family with CMT type 1A resulting from a *PMP22* point mutation. It could be speculated that additional factors, such as another locus, a unique mutation such as a trinucleotide-repeat expansion, genetic background, and environmental influences contribute to this unusual disease manifestation.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genome Database, http://www.gdb.org (for genetic maps of the markers and allele frequencies)

Hereditary Hearing Loss home page, http://alt-www.uia.ac.be/dnalab/hhh (for information on mapped genes/loci associated with deafness)

Human Cochlear EST Database, http://www.partners.org/bwh/pathology/knowngenes.html and http://www.partners.org/bwh/pathology/mapped.html

Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/Omim (for Charcot-Marie-Tooth disease [MIM 118300], CMT type 1A [MIM 118220], CMT type 1B [MIM 118200], an X-linked motor-sensory neuropathy with deafness and mental retardation [MIM 310490], autosomal recessive CMT and deafness [MIM 214370], autosomal recessive HMSN, deafness, and unusual neuropathological features [MIM 601455], and DSS [MIM 145900]

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