Clin Genet 2000: 58: 396–402
Printed in Ireland. All rights reserved

CLINICAL GENETICS
ISSN 0009-9163

Short Report

Mutational analysis and genotype/phenotype correlation in Turkish Charcot–Marie–Tooth Type 1 and HNPP patients

Bissar-Tadmouri N, Parman Y, Boutrand L, Deymeer F, Serdaroglu P, Vandenberghe A, Battaloglu E. Mutational analysis and genotype/phenotype correlation in Turkish Charcot–Marie–Tooth Type 1 and HNPP patients.
Clin Genetics 2000: 58: 396–402. © Munksgaard, 2000

The major Charcot–Marie–Tooth Type 1 (CMT1) locus, CMT1A, and Hereditary neuropathy with liability to pressure palsies (HNPP) cosegregate with a 1.5-Mb duplication and a 1.5-Mb deletion, respectively, in band 17p11.2. Point mutations in peripheral myelin gene 22 (*PMP22*), myelin protein zero (*MPZ*), and connexin 32 (*Cx32*) have been reported in CMT1, and in *PMP22* in HNPP patients without deletion. We have screened 54 CMT1 patients, of variable clinical severity, and 25 HNPP patients from Turkey, with no duplication or deletion, for mutations in the *PMP22* and *Cx32* genes. A novel frameshift mutation affecting the second extracellular domain of PMP22 was found in an HNPP patient, while a point mutation in the second transmembrane domain of the protein was detected in a CMT1 patient. Two point mutations affecting different domains of *Cx32* were identified in two CMTX patients. Another patient was found to carry a polymorphism in a non-conserved codon of the *Cx32* gene. The clinical phenotypes of the patients correlate well with the effect of the mutation on the protein.

N Bissar-Tadmouri[a], Y Parman[b], L Boutrand[c], F Deymeer[b], P Serdaroglu[b], A Vandenberghe[c] and E Battaloglu[a]

[a] Bogazici University, Department of Molecular Biology and Genetics, 80815 Bebek, Istanbul, Turkey, [b] Istanbul University, Medical School, Department of Neurology, Capa, Istanbul, Turkey, [c] Human Molecular Genetics Laboratory, Faculty of Pharmacy, University Claude Bernard Lyon 1, F-69008 Lyon, France

Key words: CMT1 – *Cx32* – frameshift mutation – HNPP – *PMP22* – point mutation – polymorphism – Turkey

Corresponding author: Esra Battaloglu, Bogazici University, Department of Molecular Biology and Genetics, 80815 Bebek, Istanbul, Turkey. Tel: +90 212 2631540/1883; fax: +90 212 2659778; e-mail: battalog@boun.edu.tr

Received 8 May 2000, revised and accepted for publication 12 July 2000

Charcot–Marie–Tooth disease (CMT) is the most common inherited peripheral neuropathy, with a total prevalence rate of 1 in 2500 (1). Clinical features of CMT1, the major form of the disease, include progressive distal muscle weakness and atrophy, often with *pes cavus* deformity. These signs are accompanied by mild sensory loss and absence of deep tendon reflexes. All patients have considerably reduced nerve conduction velocities (NCVs) (2) and segmental de- and re-myelination with typical onion-bulb formation are evident on nerve biopsies (3). Clinical onset is usually during the second or third decade of life. There is a wide range of variation in clinical severity among unrelated individuals and even among family members (4), including identical twins (5).

CMT1 is a genetically heterogeneous disorder that exhibits mostly autosomal dominant inheritance (2). The first locus for autosomal dominant CMT1, CMT1B, was mapped to chromosome 1q21-23 (6–8). The gene responsible for CMT1B codes for the major peripheral myelin glycoprotein myelin protein zero (MPZ) (9) that functions as a double adhesion molecule to hold together the myelin sheaths (10). The second locus, CMT1A, was mapped to chromosome 17p (11). CMT1A constitutes more than 70% of CMT1 cases (12). It is most often associated with a 1.5-Mb tandem duplication on chromosome 17p11.2-p12 (13), a region that contains the peripheral myelin protein 22 (*PMP22*) gene (14–17), or rarely with point mutations in the *PMP22* gene, as reviewed by Nelis et al. (18).

An X-linked dominant form of CMT1 (CMTX) was found to be associated with point mutations in connexin 32 (*Cx32*), a gap junction protein gene (19).

Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominant disorder causing variously located recurrent nerve palsies, precipitated by minor trauma.

HNPP is associated with decreased motor velocities, prolonged distal latencies, and altered sensory nerve action potentials, even in clinically non-affected areas (20). An interstitial deletion of the 1.5-Mb region, which is duplicated in the majority of CMT1A patients, was demonstrated in 84% of HNPP patients (12, 21). The description of mutations in the *PMP22* gene of some HNPP patients, as reviewed in Nelis et al. (18), demonstrated the involvement of this gene in the pathogenesis of HNPP.

In this study, we report mutations in *PMP22* and *Cx32* genes in Turkish patients with inherited peripheral neuropathy. Five sequence alterations were identified, two of which represent novel mutations, and one represents a novel polymorphism.

Patients and methods

Patients

Blood samples were obtained from 64 unrelated CMT1 patients and 39 HNPP patients diagnosed according to well-established criteria (2, 3, 22, 23). Informed consent was obtained.

Duplication/deletion analysis

Patients were screened first for the presence of the CMT1A duplication or HNPP deletion on chromosome 17p11.2. Ten duplications and 14 deletions were detected by EcoRI/SacI Southern blots hybridized with pNEA 102, a subclone of C20G2, which maps to the distal CMT1A-REP element (24). The presence of duplications or deletions was assumed by the detection of the 3.2- and 7.8-kb junction fragments in CMT1A and HNPP patients, respectively. Recombinations outside the hot-spot region were detected by visual comparison of the intensity of the hybridization signals of the EcoRI/SacI fragments from the proximal and distal CMT1A-REPs (25). These results were confirmed by dosage analysis using restriction fragment length polymorphism probes pVAW401HE and pVAW409R3a (26).

The remaining patients, without duplication (54) and deletion (25), were further screened for the presence of point mutations in the coding and exon-flanking regions of *Cx32* and *PMP22* genes. We had previously identified two novel mutations in the *MPZ* gene in this patient group (27).

Amplification and SSCP

The coding region of *Cx32* and the four coding exons of the *PMP22* gene were amplified using the primer sequences described by Bergoffen et al. (19) and Roa et al. (28), respectively. Polymerase chain reaction (PCR) products were analyzed for the presence of SSCPs on two different 0.5 × HydroLink MDE (FMC, USA) gels, one of which contained 4% glycerol. Products were visualized by silver staining.

Sequence analysis

Sequence analysis of exons 3 and 4 of *PMP22*, and amplicons 1, 2 and 3 of exon 2 of *Cx32*, was performed on purified PCR products using the same primers as for the SSCP analysis. For the automated sequencing, we used the ABI PRISM dRhodamine Terminator Cycle Sequencing Kit with AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA) on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Restriction analysis

Ten microliters of the amplified products were digested with five units of the corresponding restriction enzyme in a final volume of 15 µl. Digestions were incubated at 37°C overnight. Except for the *Nla* III digestion in P34, restriction fragments were resolved on 2% agarose gels, stained with ethidium bromide, and visualized under UV. *Nla* III restriction fragments were resolved on an 8% polyacrylamide gel (19 acrylamide:1 bisacrylamide), followed by silver staining.

Results

SSCP and subsequent sequencing analysis identified point mutations in 5 patients. Two patients, P23 and P28, were found to carry mutations in the *PMP22* gene, while 2 others, P10 and P34, had point mutations in the *Cx32* gene. In addition to these, the fifth patient was found to have a polymorphism in the *Cx32* gene (Table 1). The mutations detected were confirmed by restriction analysis. Restriction analysis was extended to 50 unrelated healthy controls. None had the variation, and thus we concluded that the mutations identified are not common polymorphisms.

Table 1. List of the *PMP22* and *Cx32* mutations presented in this study

Patient	Phenotype	Gene affected	Mutation type	Nucleotide change	Amino acid change
*P23	HNPP	*PMP22*	Frameshift	364-365delCC	P122Late stop
P28	Severe CMT1	*PMP22*	Missense	215C>T	S72L
P34	Moderate CMT1	*Cx32*	Missense	271G>A	V91M
*P10	Moderate CMT1	*Cx32*	Missense	631T>C	Y211H
P27	CMT1	*Cx32*	Silent	507C>T	D169D

*Patient with novel mutation case.

Clinical findings

Patient P23 is a 20-year-old female who complained of 4th- and 5th-finger paresthesia of the right hand that regressed spontaneously in 6 months, at age 15. She had the same complaint at age 17, but paresthesia spread to other fingers of the same hand. Since then, she had night paresthesia of feet. She also had paresthesia when leaning on her elbows and crossing legs. At the age of 17, she was found to have bilateral *pes cavus*, mild distal muscle weakness of the upper extremities with hyporeflexia and diminished vibratory sensation in the lower extremities. She also had bilateral median, ulnar and peroneal, and right radial hypoesthesia. Electrophysiology showed slowed ulnar motor NCVs (30 m/s), and prolonged distal latencies with multiple entrapment neuropathies (bilateral ulnar, median and peroneal). Radial nerve biopsy revealed a demyelinating neuropathy with tomacula in teasing.

Patient P28 is a 7-year-old male whose mother had *pes cavus* without any clinical signs. He was affected since birth and motor milestones were delayed. His examination showed *pes cavus*, distal wasting, weakness of all limbs, and areflexia. He was very ataxic and could not walk alone. Electrophysiology showed inexcitable nerves.

Patient P10 is a 21-year-old male. He had an affected male cousin and his mother and grandmother complained of hand tremor. He had hand tremor with an unsteady gait since age 13. At age 20, he was found to have *pes cavus*, horizontal nystagmus and distal muscle wasting and weakness at the lower extremities, milder distal weakness at the upper extremities, Achillean areflexia, and diminished vibratory sensation in the lower extremities with ataxia. Electromyography (EMG) showed a sensorimotor axonal neuropathy with demyelinating features. His median motor NCV was 39 m/s. His sural nerve biopsy revealed a demyelinating hypertrophic neuropathy with moderate axonal loss. His mother was found to have *pes cavus*, Achillean areflexia, and diminished vibratory sensation in her lower extremities. Her EMG showed slowed NCVs.

Patient P34 is a 22-year-old male whose mother was affected. He was found to have *pes cavus*, mild distal weakness of upper limbs, moderate distal wasting and weakness of the lower limbs, and areflexia of lower limbs. He was ataxic on walking. Electrophysiology showed slowed motor NCVs (median motor NCV is 29 m/s), prolonged distal latencies with low compound muscle action potential amplitudes, and inexcitable sensory nerves.

There was no consanguinity in any of the families, except in a parent of P28, who were distant relatives. All patients had healthy siblings, except P23.

PMP22 mutations

Patient P23, who shows an HNPP phenotype, was found to carry a novel frameshift mutation, 364-365delCC. The 2-nucleotide deletion causes a shift in the reading frame sequence, starting from codon P122, which is 39 codons before the stop codon, and results in a protein 87 amino acids longer than the wild-type protein. The mutation abolishes a restriction site of *Msp* I that normally digests the 230-bp fragment into 160 and 70 bp (Fig. 1).

A previously reported missense mutation in the *PMP22* gene associated with a severe CMT1 phenotype was found in patient P28. A C to T transition at nucleotide 215 causes substitution of leucine for serine at amino acid position 72 (S72L)

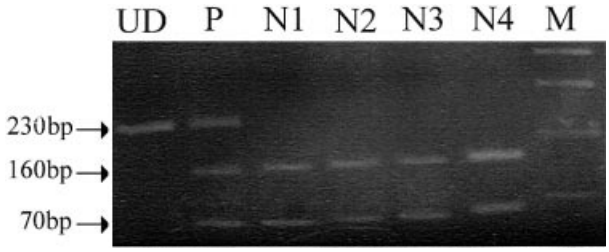

Fig. 1. Restriction analysis for patient P23. UD represents an undigested PCR product, and M is a length marker. N1, N2, N3, and N4 are PCR products from normal controls digested with *Msp* I. The digestion produces 160- and 70-bp fragments in the normal controls. The patient is heterozygous for the frameshift mutation, which abolishes the *Msp* I restriction site and leaves an undigested 230-bp fragment.

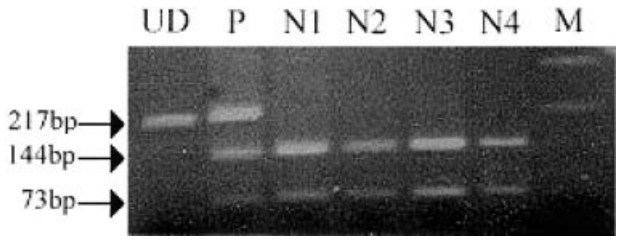

Fig. 2. Restriction analysis for patient P28. UD represents an undigested PCR product and M is a length marker. *Taq* I digestion in the patient, P, leaves an undigested 217-bp fragment representing the abolishment of the restriction site in the mutant allele. PCR fragments from the normal controls, N1, N2, N3, and N4, show complete digestion of the 217-bp fragment into 144- and 73-bp fragments.

in the second transmembrane domain of PMP22. Restriction analysis with *Taq* I leaves the 217-bp PCR fragment of the mutant allele undigested and produces 144- and 73-bp fragments in the normal allele (Fig. 2).

Connexin 32 mutations

Patient P34 was found to carry a G to A transition at nucleotide 271, leading to the substitution of methionine for valine at codon 91 (V91M). The mutation affects the second transmembrane domain of connexin 32 and creates an *Nla* III restriction site. Cutting the 438-bp long PCR fragment from the normal allele produces 116-, 96-, 66-, 59-, 55-, 36-, and 10-bp fragments. The mutation results in digestion of the 59-bp fragment into 53- and 6-bp fragments by the enzyme (Fig. 3).

Another novel mutation, a T to C transition at nucleotide 631 in the *Cx32* gene, was detected in patient P10. The mutation is predicted to result in a tyrosine to histidine substitution at codon 211

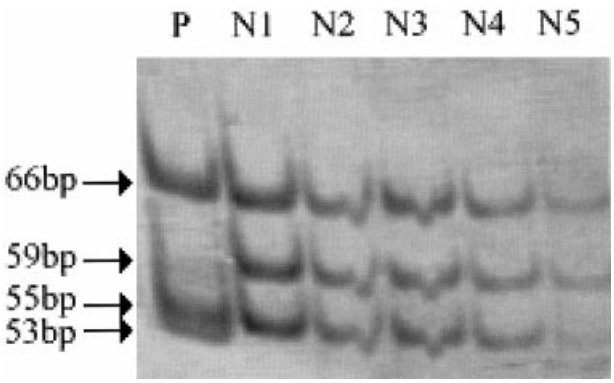

Fig. 3. Restriction analysis for patient P34. The *Nla* III digestion products from the normal controls, N1, N2, N3, N4, and N5, show 66-, 59-, and 55-bp fragments. The patient, P, however, is hemizygous for the mutation in *Cx32*, which causes the digestion of the 59-bp fragment into 53- and 6-bp fragments.

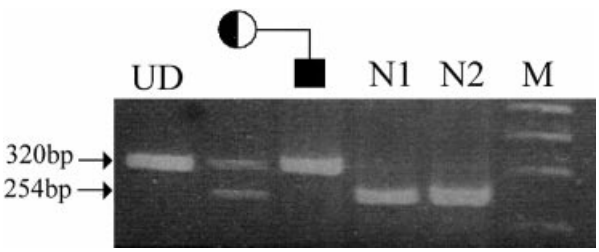

Fig. 4. Restriction analysis for patient P10. UD represents an undigested PCR product and M represents a length marker. N1 and N2 represent a 320-bp PCR product from normal controls digested with *Rsa* I resulting in a 254-bp fragment. The *Cx32* mutation in the hemizygous patient P10 (filled square) abolishes the *Rsa* I restriction site, leaving the 320-bp PCR product undigested, while the mother (half filled circle) is heterozygous for the mutation.

(Y211H). Digestion of the 320-bp-long PCR product with *Rsa* I in normal individuals produces 254- and 66-bp fragments. Restriction analysis with *Rsa* I enzyme in the patient and his mother revealed the abolishment of the restriction site by the mutation (Fig. 4).

Screening for mutations in the coding exon of connexin 32 revealed a novel polymorphism, a C to T transition at nucleotide 507, in a CMT1 patient (P27). This variant does not change the incorporation of the amino acid aspartic acid (D169D) into the protein. The 438-bp PCR fragment from the normal allele has two *Mae* II restriction sites producing three fragments of 225, 142, and 71 bp. The nucleotide change in the patient abolishes the second restriction site and produces two fragments sized 367 and 71 bp (Fig. 5). This polymorphism was not detected in 20 unrelated healthy controls.

Discussion

In this study of 64 unrelated CMT1 patients and 39 unrelated HNPP patients, the frequencies of CMT1A duplication (15.6%) and HNPP deletion (35.9%) were found to be low compared to those

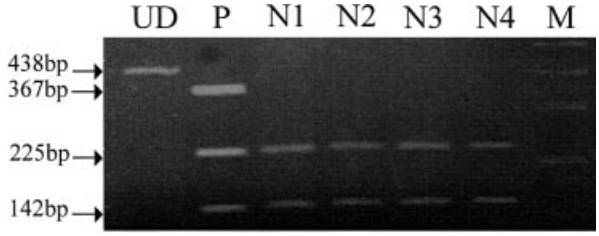

Fig. 5. Restriction analysis for the polymorphism in patient P27. UD represents an undigested PCR product and M represents a marker. Digestion with *Mae* II produces 225- and 142-bp fragments in the normal individuals, N1, N2, N3, and N4, but leaves an undigested 367-bp fragment from the polymorphic allele in the patient (P).

reported for other populations (70.7 and 84%, respectively) (12). This can be attributed to the small number of our patients. Among the patients studied, 62.5% of CMT1 and 64% of HNPP patients were sporadic cases; thus, the possibility of an acquired neuropathy in some of them cannot be ruled out. In addition, four of the CMT1 cases without any mutation have NCV values very close to the cut-off value (38 m/s), indicating that they may have an axonal type of disease (CMT2).

In all the patients presented in this study, the severity of the phenotype seems to correlate with the predicted impact of the mutation detected. PMP22 is predicted to have four transmembrane domains in which the amino acid sequence is highly conserved across species (29). PMP22 has been hypothesized to act as an adhesion molecule interacting with other PMP22 proteins in the membrane or with other adhesion molecules located in the myelin sheath (30). Alterations in expression, as in CMT1A duplication and HNPP deletion patients, or in the structure of the protein, may impair the formation and/or the maintenance of peripheral myelin.

In patient P23, who exhibits an HNPP phenotype, the 2-nucleotide deletion affects the second extracellular domain of the protein. This frameshift starting from codon P122 results in an aberrant protein that is 87 amino acids longer than the wild type and is probably non-functional. This agrees with the hypothesis that HNPP is caused either by the loss of the complete *PMP22* gene or a loss-of-function mutation in PMP22 (31–33).

Patient P28 was found to carry a missense mutation in codon 72 (S72L) of the *PMP22* gene associated with a severe CMT1 phenotype. Interestingly, the same mutation was previously found to be associated with a severe CMT1 (previously known as Dejerine Sottas Syndrome) and congenital hypomyelination neuropathy in different patients (34–38). Similarly, two other mutations in the same codon, S72W (39) and S72P (40), were reported to cause a severe CMT1 phenotype. A change in this conserved amino acid residue in the transmembrane domain, therefore, might cause a dominant negative effect or a gain of function rather than a loss of function as in HNPP patients.

In fact, most of the *PMP22* mutations are located in the putative transmembrane domains and tend to cause a more severe phenotype than that of the CMT1A duplication (18). Mutation analysis indicates the functional importance of the transmembrane domains of PMP22 and suggests that the protein acts as an anchor protein or as part of a channel, rather than as an adhesion molecule (41). However, further analysis needs to be done to shed light on the function of PMP22.

Cx32 belongs to the connexin family of channel-forming peptides. These channels are composed of two hemichannels or connexons, each composed of six identical connexin subunits arranged circularly in the plasma membrane. The four transmembrane domains of *Cx32* (M1, M2, M3, and M4) are closely packed in a ring so that helix M3 forms the wall of the channel and helices M2 and M4 are on both sides of it (42).

The two *Cx32* mutations detected in this study affect different domains of the protein and are associated with moderate CMT1 phenotypes. The Y211H mutation in patient P10 affects the third cytoplasmic domain of the protein towards the carboxy terminus and is very close to the membrane–cytoplasm boundary. This mutation introduces a positive charge and may interfere with gating stimulus control (43). A Y211X nonsense mutation associated with a moderate phenotype has been reported (44), causing the synthesis of a premature and therefore presumably non-functional protein.

The mutation in patient P34 affects the second transmembrane domain of *Cx32* only slightly since a nonpolar amino acid is substituted by another nonpolar residue. The alteration may cause incorrect alignment of two exons, or lead to the inability to form a functional gap junction between two exons. Frameshift mutations in this domain were also reported to lead to a moderate phenotype because a truncated protein is not incorporated into the membrane; thus, formation of gap junctions by other connexins are allowed (45, 46).

Genotype/phenotype correlation in CMT1 is very important in assessing the function and the functional domains of the myelin proteins. Analysis of the mutations in the connexin 32 gene would reveal which parts of the molecule are important for the proper formation of functional gap junctions. Point mutations in *PMP22* can result in CMT1 phenotypes of different severity, or HNPP phenotypes, depending on the effect of the mutation on the structure and function of the protein. Determination of the mutational spectrum of peripheral myelin genes, coupled with functional studies of the mutations, are required to ascertain the mechanism through which different abnormal myelin proteins and, consequently, myelin dysfunction can lead to variable expression and often to late-onset disease.

Acknowledgements

This work was supported by grants from Bogazici University Research Fund (OOM102), TUBITAK (SBAG-2200), and Association Francaise contre les Myopathies (AFM). The group in Bogazici University is a member of the

European CMT Consortium. The laboratory of Human Molecular Genetics in Lyon is a member of the European CMT Consortium sponsored by the EU Biomed grants CT961614 and CT 960055.

References

1. Skre H. Genetic and clinical aspects of Charcot–Marie–Tooth disease. Clin Genet 1974: 6: 98–118.
2. Harding AE, Thomas PK. The clinical features of hereditary motor and sensory neuropathy types I and II. Brain 1980: 103: 259–280.
3. Dyck PJ. Inherited neuronal degeneration and atrophy affecting peripheral motor, sensory, and autonomic neurons. In: Dyck PJ, Thomas PK, Lambert EH, eds. Peripheral neuropathy, 2nd ed. Philadelphia and London: WB Saunders, 1975: 825–867.
4. Kaku DA, Parry GJ, Malamut R, Lupski JR, Garcia C. Nerve conduction studies in Charcot–Marie–Tooth polyneuropathy associated with a segmental duplication of chromosome 17. Neurology 1993: 43: 1806–1808.
5. Garcia CA, Malamut RE, England JD, Parr GS, Liu P, Lupski JR. Clinical variability in two pairs of identical twins with the Charcot–Marie–Tooth type 1A duplication. Neurology 1995: 45: 2090–2093.
6. Bird TD, Ott J, Giblett ER. Evidence for linkage of Charcot–Marie–Tooth neuropathy to the duffy locus on chromosome 1. Am J Hum Genet 1982: 34: 388–394.
7. Lebo RV, Chance PF, Dyck PJ, Bird TD. Chromosome 1 Charcot–Marie–Tooth disease (CMT 1B) locus in the Fcg receptor gene region. Hum Genet 1991: 88: 1–12.
8. Lebo RV, Lynch ED, Wiegant J, Moore K, Trounstine M, van der Ploeg M. Multicolor fluorescence in situ hybridization and pulsed field electrophoresis dissect CMT1B gene region. Hum Genet 1991: 88: 13–20.
9. Pham-Dinh D, Fourbil Y, Blanquet F. The major peripheral myelin protein zero gene: structure and localization in the cluster of Fcg receptor genes on human chromosome 1q21.3-q23. Hum Mol Genet 1993: 2: 2051–2054.
10. Fiblin MT, Walsh FS, Trapp BD, Pizzey JA, Tennekoon GI. Role of myelin P0 protein as a homophilic adhesion molecule. Nature 1990: 344: 871–872.
11. Vance JM, Barker D, Yamaoka LH. Localization of Charcot–Marie–Tooth disease type 1A (CMT1A) to chromosome 17p11.2. Genomics 1991: 9: 623–628.
12. Nelis E, Van Broeckhoven C. Estimation of the mutation frequencies in Charcot–Marie–Tooth disease type 1 and hereditary neuropathy with liability to pressure palsies: a European collaborative study. Eur J Hum Genet 1996: 4: 25–33.
13. Lupski JR, Oca-Luna RM, Slaugenhaupt S. DNA duplication associated with Charcot–Marie–Tooth disease type 1A. Cell 1991: 66: 219–232.
14. Valentijn LJ, Bolhuis PA, Zorn I. The peripheral myelin protein PMP-22/Gas-3 is duplicated in Charcot–Marie–Tooth disease type 1A. Nature Genet 1992: 1: 166–170.
15. Timmerman V, Nelis E, Van Hul W. The peripheral myelin protein gene PMP-22 is contained within the Charcot–Marie–Tooth Disease type 1A duplication. Nature Genet 1992: 1: 171–175.
16. Matsunami N, Smith B, Ballard L. Peripheral myelin protein-22 gene maps in the duplication in chromosome 17p11.2 associated with Charcot–Marie–Tooth 1A. Nature Genet 1992: 1: 176–179.
17. Patel P, Roa BB, Welcher AA. The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot–Marie–Tooth disease type 1A. Nature Genet 1992: 1: 159–165.
18. Nelis E, Haites N, Van Broeckhoven C. Mutations in the peripheral myelin genes and associated genes in inherited peripheral neuropathies. Hum Mutat 1999: 13: 11–28.
19. Bergoffen J, Scherer SS, Wang S. Connexin mutations in X-linked Charcot–Marie–Tooth. Science 1993: 262: 2039–2042.
20. Beshe F, Buchthal F, Carlsen F, Knappeis GG. Hereditary neuropathy with liability to pressure palsies: electrophysiological and histopathological findings. Brain 1972: 95: 777–794.
21. Chance PF, Alderson MK, Leppig KA. DNA deletion associated with hereditary neuropathy with liability to pressure palsies. Cell 1993: 72: 143–151.
22. De Visser M. Diagnostic criteria for aurosomal dominant hereditary motor and sensory neuropathy type 1A. Neuromuscul Disord 1993: 3: 77–79.
23. Dubourg O, Mouton P, Brice A, Le Guern E, Bouche P. Guidelines for diagnosis of hereditary neuropathy with liability to pressure palsies. Neuromuscul Disord 2000: 10: 206–208.
24. Pentao L, Wise CA, Chinault AC, Patel PI, Lupski JR. Charcot–Marie–Tooth type 1A duplication appears to arise from recombination at repeat sequences flanking the 1.5 Mb monomer unit. Nature Genet 1992: 2: 292–300.
25. Chance PF, Abbas N, Lensch MW. Two autosomal dominant neuropathies result from reciprocal DNA duplication/deletion of a region on chromosome 17. Hum Mol Genet 1994: 3: 223–228.
26. Raeymaekers P, Timmerman V, Nelis E. Estimation of the size of the chromosome 17p11.2 duplication in Charcot–Marie–Tooth neuropathy type 1a (CMT 1a). J Med Genet 1992: 29: 5–11.
27. Bissar-Tadmouri N, Gulsen-Parman Y, Latour P, Deymeer F, Serdaroglu P, Vandenberghe A, Battaloglu E. Two novel mutations in the MPZ gene coding region in Charcot–Marie–Tooth type 1 patients of Turkish origin: S54P, [I30del: GVYI29ins]. Hum Mutat 1999: 14: 449.
28. Roa BB, Garcia CA, Suter U. Charcot–Marie–Tooth disease type 1A: association with a spontaneous point mutation in the PMP22 gene. N Engl J Med 1993: 329: 96–101.
29. Hayasaka K, Himoro M, Nanao K. Isolation and sequence determination of cDNA encoding PMP-22 (PAS II/SR13/Gas-3) of human peripheral myelin. Biochem Biophys Res Commun 1992: 186: 827–831.
30. Suter U, Snipes GJ. Peripheral myelin protein 22: facts and hypotheses. J Neurosci Res 1995: 40: 145–151.
31. Pareyson D, Taroni F. Deletion of the PMP22 gene and hereditary neuropathy with liability to pressure palsies. Curr Opin Neurology 1996: 9: 348–354.
32. Vallat J-M, Sindou P, Preux P-M. Ultrastructural PMP22 expression in inherited demyelinating neuropathies. Ann Neurol 1996: 39: 813–817.
33. Schenone A, Nobbio L, Mandich P. Underexpression of messenger RNA for peripheral myelin protein 22 in hereditary neuropathy with liability to pressure palsies. Neurology 1997: 48: 445–449.
34. Roa BB, Dyck PJ, Marks HG, Chance PF, Lupski JR. Dejerine-Sottas syndrome associated with point mutation in the peripheral myelin protein 22 (PMP22) gene. Nat Genet 1993: 5: 269–273.
35. Ionasescu VV, Searby C, Greenberg SA. Dejerine-Sottas disease with sensorineural hearing loss, nystagmus, and peripheral facial nerve weakness: de novo dominant point mutation of the PMP22 gene. J Med Genet 1996: 33: 1048–1049.

36. Ionasescu VV, Ionasescu R, Searby C, Neahring R. Dejerine-Sottas disease with de novo dominant mutation of the PMP22 gene. Neurology 1997: 45: 1766–1767.
37. Marques W Jr, Thomas PK, Sweeney MG, Carr L, Wood NW. Dejerine-Sottas neuropathy and PMP22 point mutations: a new base pair substitution and a possible "hot spot" on Ser72. Ann Neurol 1998: 43: 680–683.
38. Simonati A, Fabrizi GM, Pasquinelli A. Congenital hypomyelination neuropathy with Ser72Leu substitution in PMP22. Neuromusc Disord 1999: 9: 257–261.
39. Tyson J, Ellis D, Fairbrother U. Hereditary demyelinating neuropathy of infancy. A genetically complex syndrome. Brain 1997: 120: 47–63.
40. Haites NE, Nelis E, Van Broeckhoven C. 3d workshop of the European CMT consortium: 54th ENMC international workshop on genotype–phenotype correlations in Charcot–Marie–Tooth type 1 and hereditary neuropathy with liability to pressure palsies, 28–30 November 1997, Naarden, The Netherlands. Neuromuscul Dis 1998: 8: 591–603.
41. Ikegami T, Ikeda H, Aoyama M. Novel mutations of the peripheral myelin protein 22 gene in two pedigrees with Dejerine-Sottas disease. Hum Genet 1998: 102: 294–298.
42. Milks LC, Kumar NM, Houghten R, Unwin N, Gilula NB. Topology of the 32-Kd liver gap junction protein determined by site-directed antibody localizations. EMBO J 1988: 7: 2967–3975.
43. Fairweather N, Bell C, Cochrane S. Mutations in the connexin 32 gene in X-linked dominant Charcot–Marie–Tooth disease (CMTX1). Hum Mol Genet 1994: 3: 29–34.
44. Tan CC, Ainsworth PJ, Hahn AF, MacLeod PM. Novel mutations in the connexin 32 gene associated with X-linked Charcot–Marie–Tooth disease. Hum Mutat 1996: 7: 167–171.
45. Sorour E, Upadhyaya M. Mutation analysis in Charcot–Marie–Tooth disease type 1 (CMT1). Hum Mutat 1998: S1: S242–S247.
46. Bone LJ, Scherer SS, Balice-Gordon RJ, Paul DL, Fischbeck KH. The disease mechanism for X-linked Charcot–Marie–Tooth disease. Am J Hum Genet 1995: 57 (Suppl): 1366.