HUMAN MUTATION 15:340–347 (2000)

RESEARCH ARTICLE

Screening for Mutations in the Peripheral Myelin Genes *PMP22, MPZ* and *Cx32* (*GJB1*) in Russian Charcot-Marie-Tooth Neuropathy Patients

Irina V. Mersiyanova,[1] Sookhrat M. Ismailov,[1] Alexandr V. Polyakov,[1] Elena L. Dadali,[2] Valeriy P. Fedotov,[3] Eva Nelis,[4] Ann Löfgren,[4] Vincent Timmerman,[4] Christine Van Broeckhoven,[4] and Oleg V. Evgrafov[1]*

[1]*Laboratory of DNA Diagnostics, Research Centre for Medical Genetics, Moscow, Russia*
[2]*Genetic Counseling Department, Research Centre for Medical Genetics, Moscow, Russia*
[3]*Genetic Counseling Department, Diagnostic Centre, Voronezh, Russia*
[4]

CMT neuropathy is known to be genetically heterogeneous, and to date three disease-causing myelin genes have been identified: peripheral myelin protein 22 (*PMP22*) on chromosome 17p11.2 (MIM# 601097); myelin protein zero (*MPZ/P0*) on 1q22-23 (MIM# 159440); and connexin 32 (*Cx32/GJB1*) on Xq13.1 (MIM# 304040). They correspond to the genetic loci CMT1A, CMT1B, and CMT1X, respectively [De Jonghe et al., 1997]. Mutations implicating the *PMP22*, *MPZ*, and *Cx32* genes are mainly responsible for peripheral demyelinating neuropathies such as CMT1, Dejerine-Sottas syndrome (DSS), congenital hypomyelination (CH), and hereditary neuropathy with liability to pressure palsies (HNPP). At least eleven other genetic loci for CMT and related neuropathies, for which the genes have not yet been identified, are localised on different chromosomes [De Jonghe et al., 1997].

The most frequent cause of CMT1 disease is a 1.5 Mb tandem duplication on chromosome 17p11.2, known as the CMT1A duplication [Lupski et al., 1991; Raeymaekers et al., 1991]. This duplication is caused by an unequal crossover event between two homologous repetitive elements which flank the 1.5 Mb region [Reiter et al., 1996]. The CMT1A duplication comprises the *PMP22* gene [Matsunami et al., 1992; Patel et al., 1992; Timmerman et al., 1992; Valentijn et al., 1992]. Deletion of the 1.5 Mb region, including *PMP22*, causes HNPP [Chance et al., 1993]. Changing the dosage of PMP22 results in progressive alteration of the myelin sheath of peripheral nerves [Lupski et al., 1992]. It is interesting to note that point mutations in *PMP22* also result in a CMT1, DSS or HNPP phenotype [Nelis et al., 1999]. The *MPZ* gene, which forms the major component of myelin, codes for a self-adhesion molecule [Lemke, 1988]. Mutations in *MPZ* lead to CMT1, DSS, and CH phenotypes. Mutations in *MPZ* have also been found in some CMT patients with slightly reduced or normal NCVs, which is indicative of a CMT2 phenotype [Marrosu et al., 1998; De Jonghe et al., 1999]. *Cx32* molecules form gap-junction channels in the peripheral nervous system which provide exchange of small molecules between neighbouring myelin layers [Bennet et al., 1991]. *Cx32* mutations lead to a CMT1 phenotype in most cases [Nelis et al., 1999], but *Cx32* mutations usually cause a more severe phenotype in men than in women because the *Cx32* gene is localised on the X-chromosome [Birouk et al., 1998]. Mutations in the gene coding for the early growth response 2 (*EGR2*) transcription factor (MIM# 129010) were recently found in patients with a CMT1, DSS, or CH phenotype [Warner et al., 1998; Timmerman et al., 1999].

We performed a 1.5 Mb CMT1A duplication and myelin gene mutation screening in CMT patients of Russian origin diagnosed as CMT1, CMT2, or with an unspecified CMT phenotype. We also screened three HNPP families for the presence of the 1.5 Mb HNPP deletion in 17p11.2. We performed an analysis to determine whether the disease-causing mutations and the mutation frequencies are specific for the Russian population. We also estimated the number of CMT1 patients without mutations in the known genes in order to determine the role of unknown genes in the CMT1 pathogenesis.

MATERIALS AND METHODS

Patient Samples

We performed a mutation screening in 174 unrelated CMT patients of Russian origin. One hundred and eight CMT1 patients were investigated, 55 at the Research Centre for Medical Genetics in Moscow, and 53 at the Genetic Counseling Department of the Voronege Diagnostic Centre in Voronege. The CMT1 diagnosis was based on the following criteria: slow, progressive, symmetrical muscle wasting and weakness predominantly in the distal part of the lower limbs; absent or decreased tendon reflexes; reduced motor median NCV's ($\leq$ 38 m/s); and obvious sensory disturbances with different degrees of sensory loss, including pain deficit. Seventy patients had a positive family history of the disease, and 22 were sporadic, with both parents healthy. Sixteen cases were considered to be isolated because one or both parents were not clinically examined, or reliable information on the family history was not available. Sixty-eight familial cases were apparently autosomal dominant or X-linked, and two cases were recessive. We also investigated a group of 32 CMT2 cases. For the diagnosis of these patients we used the clinical guidelines of the European CMT Consortium [De Jonghe et al., 1998]. In addition, 34 patients had an unspecified type of CMT because electrophysiological or histopathological data were not available.

CMT1A Duplication Screening

The presence or absence of 1.5 Mb CMT1A duplication or HNPP deletion was analysed using six $(CA)_n$ repeat markers localised in the duplication/deletion region (Table 1). Two multiplex amplification systems (MPA) were developed: MPA1

TABLE 1. Multiplex Amplification System (MPA) for DNA Diagnosis of the CMT1A Duplication and HNPP Deletion

	Marker	H	Size (bp)	Ta	Primer sequences
MPA1	D17S122	74%	153–167	57°C	F: AGAACCACAAAATGTCTTGCATTC R: GGCCAGACAGACCAGGCTCTGC
	D17S921	73%	109–127		F: GTGTTGTATTAGGCAGAGTTCTCC R: CACCATAATCATGTCAGACAATCC
MPA2	D17S955	45%	165–173	58°C	F: GGTTGGGTGTCCTTGGCCTAC R: ACTGGTGCATCCATGAGCATGC
	D17S2839	55%	123–143		F: CAACAACAGCGAAACTCTGTCTC R: AGACCCTGGAAGATCAACTACC
	D17S261	47%	96–110		F: CTAGGCACTGAAGCCAGGAAG R: TTCTGGAAACCTACTCCTGAGC
	D17S1358	74%	122–134	57°C	F: AGCACCATGCCGGGCCACAC R: AGATGGATAAGATGATCATGTTAC

H, heterozygosity; Ta, annealing temperature; F, forward primer; R, reverse primer.

includes markers D17S122 (RM11-GT) and D17S921 (AFM191xh12); MPA2 contains markers D17S955 (AFM317yg1b), D17S839 (AFM-200yb12), and D17S261 (Mfd41). The sixth marker, D17S1358 (133C4ac1), was analysed separately. We used previously published primer sequences [Lupski et al., 1991] for D17S122 PCR amplification. Primer sequences for the other markers in MPA1 and MPA2 were chosen to generate PCR products that can be well separated in the multiplex systems. PCR annealing temperatures were 57°C for MPA1 and D17S1358, and 58°C for MPA2. The PCR fragments were separated on a 8% PAGE gel (ratio of acrylamide to methylen-bisacrylamide by weight = 29:1.3), using a 1× TBE buffer, 240–260V at room temperature. The gel was stained with ethidium bromide (0.1 μg/ml) and UV-photographed (312 nm). The presence of three alleles, or dosage differences between alleles of each marker, was defined by visual examination. For accurate estimation of allele dosage quantity, we used fluorescence labeled primer molecules for markers D17S122 and D17S921. Denatured PCR products were loaded on a 6% PAGE gel and electrophoresed on the automated DNA ALF™ sequencer (Pharmacia, Uppsala, Sweden) according to the manufacturer's protocol. The fragment analysis was performed with the Fragment Manager computer program (Pharmacia, Uppsala, Sweden). In the non-informative cases, the presence of the 3.2 kb CMT1A duplication-specific junction fragment was screened using the CMT1A-REP probe pLR7.8 on *Eco*RI/*Sac*I Southern blots, as described previously [Timmerman et al., 1997].

Screening of Myelin Gene Mutations

Intronic primers were used for PCR amplification of *MPZ* exons 1, 2, 3, and 6, and *MPZ* exons 4 and 5 together with intron 4. For PCR amplification of the *Cx32* coding region we used primers flanking exon 2. Sequences for the primer molecules, as well as PCR conditions for *MPZ* and *Cx32,* are available upon request. PCR amplification of four exons of *PMP22* was carried out as described previously [Roa et al., 1993b].

Twenty genomic DNA samples were analysed for the presence of point mutations in the *PMP22, MPZ,* and *Cx32* genes in the Molecular Genetics Laboratory at the University of Antwerp, Belgium, using primers and PCR conditions published elsewhere [Roa et al., 1993b; Nelis et al., 1994; Nelis et al., 1996b; Nelis et al., 1997b].

Single strand conformation polymorphism (SSCP) analysis was used as the first screening method to predict point mutations in *PMP22, MPZ,* and *Cx32*. Because this method is the most effective for short DNA fragments, long PCR products were fragmented with a set of restriction endonucleases before SSCP analysis (*Sma*I for *MPZ* exon 2; *Hha*I or *Acy*I for *MPZ* exon 3; *Sty*I for *MPZ* exons 4 and 5; and *Msp*I or *Bst*XI for *Cx32* exon 2). For SSCP analysis we performed the following alkaline denaturation of PCR products: an aliquot of 15 μl of each amplified PCR product (or PCR product after restriction endonuclease digestion) was mixed with 5 μl of 0.5 M NaOH and 0.5 μl of 0.5 M EDTA, then heated at 42°C for 10 min. Each amplification product was combined with four μl of loading buffer (95% formamide, 0.5% bromophenol blue, and 0.5% xylolcyanol), loaded on non-denaturing 8% PAGE gel (with or without 5% glycerol), then electrophoresed in 0.5x TBE at 110 V at room temperature for 18–24 hr. The gel was stained with silver nitrate using the Silver sequence kit according to the standard protocol (Promega, Madison, WI). PCR products showing a change in electrophoretic mobility were sequenced using forward and reverse primers. In the DNA Diagnostic Laboratory of the Moscow Re-

search Center of Medical Genetics, DNA sequencing of *MPZ* exons 3 and 6, and of *Cx32* exon 2, was performed with the automated ALF™ DNA sequencer (Pharmacia, Uppsala, Sweden) using fluorescein-labeled primers. Part of the DNA samples were sequenced in the Molecular Genetics Laboratory at the University of Antwerp, Belgium, using the ABI Prism™ dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit and the ABI 310 DNA sequencer, according to manufacturer's protocols (Applied Biosystems, Foster City, CA).

RESULTS

CMT1A Duplication and HNPP Deletion Screening

The CMT1A duplication study was performed using 6 $(CA)_n$ repeat markers from within the 1.5 Mb region in 17p11.2-p12. We considered the presence of the CMT1A duplication in a CMT patient if at least for one DNA marker three distinct alleles were detected, or if clear dosage differences were seen for two different markers. Dosage analysis can be complicated in practice when the difference in length between two $(CA)_n$ repeat alleles is only two nucleotides. In this case, the stutter effect results in an non-specific synthesis of DNA fragments. The non-specific peaks from the larger allele might affect the dosage of the shorter allele. Thus, patients presenting two adjacent alleles were considered as non-informative for such $(CA)_n$ repeat marker. This approach can be more informative if several patients and relatives from the same family are investigated. Taking all these considerations into account, only 2/108 (1.9%) CMT1 patients were non-informative for this duplication screening method. Fourteen CMT1 patients, including the two non-informative cases, were also analysed for the presence of the CMT1A duplication-specific junction fragment using the CMT1A-REP marker pLR7.8 on *Eco*RI/*Sac*I Southern blots. The CMT1A-REP analysis confirmed the results of the microsatellite analysis in eleven CMT1A duplication cases, and excluded the duplication in the two non-informative cases.

We detected 58 CMT1A duplications (53.7%) among the 108 informative CMT1 patients. In the three sporadic cases, a de novo CMT1A duplication of paternal origin was found. Duplications were also found in seven additional patients with clinically and electrophysiologically healthy parents, although the genomic DNA of the parents was not available for testing. These results lead to a de novo duplication frequency in the Russian cohort between 5.2% (3/58) and 17.2% (10/58). We found one CMT1A duplication in the patient group with an unspecified CMT type, but did not detect the CMT1A duplication in the CMT2 patient group.

We detected the 1.5 Mb HNPP deletion in 17p11.2 in all patients in the three HNPP families. Affected individuals with the HNPP deletion were hemizygous for all polymorphic markers tested with our multiplex PCR system (MPA1 and MPA2), with all alleles inherited from the healthy parent. In all three HNPP families, paternity or maternity was confirmed by the analysis of nine additional highly polymorphic DNA markers: THO1, F13B1, MCT112, D18S57, D9S290, D1S2667, D11S4127, F13A1, D7S435.

Mutation Screening of Myelin Genes

SSCP analysis for the *MPZ* and *Cx32* genes was carried out in all 115 non-CMT1A duplicated cases. SSCP for *PMP22* was performed for all 50 clinically and electrophysiologically diagnosed non-duplicated CMT1 patients. PCR products with altered electrophoretic mobility were sequenced. The following steps were taken to increase the sensitivity of our SSCP mutation screening process: all fragments were analysed using two different SSCP conditions (PAGE with or without glycerol); and we analysed relatively long PCR products in at least two different ways: digestion of the PCR product with two different restriction enzymes, and SSCP analysis of the digested and undigested DNA fragment. As a control to our procedure, we sequenced a considerable number of DNA samples without altered SSCP patterns, and no additional mutations were found.

The mutations are shown in Table 2. Most of them are single-base changes resulting in amino acid substitutions. Thirteen distinct missense mutations (*MPZ*: Arg98His, Asp134Glu, and Ile135Thr; *Cx32*: Gln80Arg, Met93Val, Arg107Trp, Arg142Trp, Arg164Gln, Arg183His, Glu186Lys, and Glu208Lys; *PMP22*: Thr118Met, and Leu147Arg) have been described previously [Nelis et al., 1999]. These mutations are listed in the mutation database of inherited peripheral neuropathies (http://molgen-www.uia.ac.be/CMTMutations/). Among the mutations in *Cx32*, Arg164Gln was found in two unrelated Russian CMT1 patients, and Arg107Trp was found in three unrelated CMT patients. Eight mutations in these myelin protein genes were not described previously. Three of them are missense mutations occurring in the third exon of the *MPZ* gene: Asp134Gly, Lys138Asn, and Thr139Asn. Four novel mutations were found in *Cx32* exon 2:

TABLE 2. Mutations in *MPZ, Cx32* and *PMP22* in CMT Patients From Russia

Gene	Exon	Domain	Nucleotide alteration	Amino acid alteration	Restriction site change	Inheritance	Phenotype
MPZ	3	EC	c.293G>A	Arg 98His	–Hha I	Dominant	CMT
	3	EC	c.401A>G	Asp134Gly*	+BstN I	De novo	CMT1
	3	EC	c.402C>A	Asp134Glu	–	Autosomal dominant	CMT1
	3	EC	c.404T>C	Ile135Thr	–	Dominant	CMT1
	3	EC	c.414G>C	Lys138Asn*	–	Dominant	CMT1
	3	EC	c.416C>A	Thr139Asn*	–Mnl I	Autosomal dominant	CMT1
	6	IC	c.684C/T	Ser228Ser	+Rsa I	Polymorphism	7 cases
Cx32	2	TM1	c.59T>A;c.61G>A	Ile20Asn,Gly21Ser*	–Hae III	Dominant	CMT1
	2	TM1	c.100A>G	Met34Lys*	–Nla III	Dominant	CMT1
	2	TM2	c.235C>T	Leu79Leu	–Pst I	Polymorphism	3 cases
	2	TM2	c.239A>G	Gln80Arg	–Alu I	Dominant	CMT
	2	TM2	c.268C>G	Leu90Val*		Isolated	CMT
	2	IC	c.277A<G	Met93Val	BstX I	Isolated	CMT
	2	IC	c.319C>T	Arg107Trp	–	Dominant	CMT
	2	TM3	c.424C>T	Arg142Trp	–Msp I	Dominant	CMT1
	2	EC2	c.491G>A	Arg164Gln	+Alu I	Isolated	CMT1
	2	EC2	c.548G>A	Arg183His	–	Isolated	CMT1
	2	EC2	c.556G>A	Glu186Lys	–	Isolated	CMT2
	2	TM4	c.579C>G	Phe193Leu*	–	Dominant	CMT1
	2	C	c.622G>A	Glu208Lys	+HaeI, +Bal I	Dominant	CMT1
PMP22	1	TM1	c.73-78 del GTCAGC	ValSer25-26del*		Isolated	CMT1
	4	TM3	c.353C>T	Thr118Met	+NlaIII	Polymorphism	CMT1
	4	TM4	c.440T>G	Leu147Arg	–	Dominant	CMT1

EC, extracellular domain; IC, intracellular domain or loop; TM, transmembrane domain; C, C-terminal domain; CMT, unspecified type of CMT.
*Previously unpublished mutations submitted to the mutation database for inherited peripheral neuropathies (IPNMDB) at http://molgen-www.uia.ac.be/CMTMutations/

Ile20Asn/Gly21Ser, Met34Lys, Leu90Val, and Phe193Leu. In *PMP22* exon 1 we found a novel 6 bp in-frame deletion of codons 25 and 26 which caused the loss of the amino acids Val and Ser.

SSCP and DNA sequencing analysis of the myelin genes also revealed silent mutations in *MPZ* exon 6 (Ser228) and *Cx32* exon 2 (Leu79). The Thr118Met substitution in *PMP22* was initially described as a recessive mutation [Roa et al., 1993a], but there is evidence that this substitution is a rare allele of a diallelic polymorphic site [Nelis et al., 1997a].

DISCUSSION

In this study we performed the mutation screening of a group of 108 clinically and electrophysiologically diagnosed CMT1 patients from Russia (Table 3). Study of this group of CMT1 patients allows comparison of the CMT1 mutation spectrum in Russia with CMT1 findings from other parts of Europe. In all European population studies, the 1.5 Mb tandem CMT1A duplication is the most frequent disease-causing mutation. The mutation frequency varies between 49% and 80% [Bort et al., 1994; Mostacciuolo et al., 1995; Nelis and Van Broeckhoven, 1996a; Silander et al., 1998]. In the 108 informative unrelated Russian CMT1 patients, we found 58 (53.7%) CMT1A duplications. This duplication frequency is consistent with other European population data in CMT1 [Nelis and Van Broeckhoven, 1996a].

The relative frequency of de novo CMT1A duplications (5.2%–17.2%) is similar to frequencies

TABLE 3. Overview of Mutations in CMT and HNPP Patients From Russia

	Total of unrelated patients	CMT1A-duplication	HNPP-deletion	Uninformative	*Cx32*	*MPZ*	*PMP22*
CMT1	108	58	0	0	8	5	2
CMT2	32	0	0	3	1	0	nd
CMT	34	1	0	1	6	1	nd
HNPP	3	nd	3	0	nd	nd	nd
Total	177	59	3	4	15	6	2

nd, not determined.

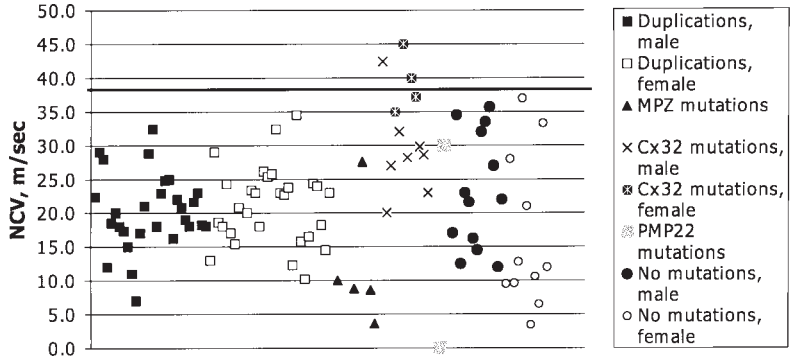

FIGURE 1. Median motor nerve conduction velocity (NCV) in CMT patients with different mutations. Only one patient from each family is presented, except in *Cx32* cases, where NCVs for one male and one female are shown, if available. Horizontal bold line indicates cut-off value of NCV for CMT1 (38 m/s). NCVs of CMT2 patients are not shown.

reported in other ethnic populations [Hoogendijk et al., 1992; Blair et al., 1996; Nelis and Van Broeckhoven, 1996a]. In the Russian CMT1 patients, we found eight *Cx32* mutations (7.4%), five *MPZ* mutations (4.6 %), and two *PMP22* mutations (1.8 %). In this study we did not perform a mutation screening of EGR2. From previous mutation screening studies we know *Cx32* mutations are almost equally distributed along the coding exon 2 [De Jonghe et al., 1997; Nelis et al., 1999]. In our Russian CMT1X patients we found a similar distribution. Arg164Gln was found twice, and Arg107Trp was found three times in our cohort.

A double missense mutation in *Cx32* (Ile20Asn/Gly21Ser) was found in a male CMT1 patient and in three relatives. The amino acids Ile20 and Gly21 are neutral with non-polar side chains, and the amino acids in the mutated variant (Asn20 and Ser21) belong to the neutral amino acids with polar side chains. Since it seems doubtful that each codon mutation is a rare variant of a normal functional Cx32 gap-junction protein, a simultaneous double mutation seems more probable in this particular case. Only a few double-codon mutations have been described in *EGR2* [Warner et al., 1998], *MPZ* [Warner et al., 1996], and *Cx32* [Silander et al., 1998].

We found six *MPZ* missense mutations within exon 3. The majority of the published mutations were also found in exon 3 [Nelis et al., 1999], suggesting that extracellular domain of MPZ is essential for MPZ functioning. One of the mutations, Asp134Gly, appears de novo and results in a CMT1 phenotype. Only a small number of de novo mutations have been found in the *MPZ* gene, most of them resulting in a DSS phenotype [Nelis et al., 1999].

We detected two distinct *PMP22* mutations: the Leu147Arg mutation replacing a neutral amino acid with non-polar side chain into a basic one; and an in-frame deletion of two amino acids, Val and Ser, at codons 25 and 26. Both mutations are located in the transmembrane domains and lead to a CMT1 phenotype. The consequence of *PMP22* gene alterations on protein structure, localisation, and function is unknown. However, mutations occurring in transmembrane regions could block transport or incorporation of the PMP22 protein into the plasma membrane [Naef and Suter, 1999; Tobler et al., 1999].

Among the 35 unrelated CMT1 patients without mutations in *PMP22*, *MPZ*, or *Cx32*, six were autosomal dominant as revealed by pedigree analysis. Further, there were eight dominant cases in which X-linked inheritance could not be excluded, since male-to-male transmission was not observed. Autosomal dominant families unlinked to known CMT1 loci were previously described and designated CMT1C [Chance et al., 1990]. Taking into account the rare *EGR2* mutations [Warner et al., 1998], the prevalence of unlinked CMT1 is approximately 32% (35/108). We conclude that the non-duplication CMT1 cases (35/50) are caused by mutations in still unidentified genes.

In our cohort of 35 CMT1 patients with an unknown mutation, two families were recessive and eleven patients had clinically healthy parents. We had no reliable information about family history in eight cases. Of these 21 cases a de novo dominant mutation or recessive mutation in unidentified genes is possible. The prevalence of autosomal recessive CMT1 can differ considerably in different populations, but recessive cases are hard to distinguish from dominant de novo mutations or germline mosaicism, which was recently reported in the literature [Takashima et al., 1999].

DNA investigation allows correlation of the genotype with the phenotype (Fig. 1). The average NCV value for the motor median nerve in our CMT1A duplication patients was 20 m/s. Patients with *Cx32* mutations revealed a sex-specific picture. The male and female average NCVs were 29.4

and 39.3 m/s, respectively. The mean NCV of CMT1X male patients was higher when compared to values in male CMT1A duplication patients. The two heterozygous CMT1X females had NCVs higher than 38 m/s, which is within the range of CMT2. One isolated male patient, diagnosed as CMT2 with NCVs of 42.4 m/s, had a *Cx32* mutation (Glu186Lys). The patient's mother considered herself to be unaffected, but she could not be examined. This Glu186Lys mutation which was reported previously in several CMT1 patients [Nelis et al., 1999]appears to be de novo.

Patients with *MPZ* mutations had very low NCVs, an average of 8.2 m/s. Two patients in the same family with the Thr139Asn *MPZ* mutation had higher NCVs: the 32-year-old male patient had NCVs of 27.6 m/s, and his affected mother had NCVs of 45.2 m/s. The Thr and Asn amino acids belong to the same group of uncharged molecules with small polar side chains. It is possible that the Thr139Asn substitution slightly modifies the adhesion properties of the *MPZ* molecule, resulting in a milder phenotype and highly variable NCVs. Highly variable NCVs within the range of CMT2 (> 38 m/s) are associated with *MPZ* mutations Thr124Met [De Jonghe et al., 1999] and Ser44Phe [Marrosu et al., 1998]. These *MPZ* mutations result in non-conservative amino acid changes. The molecular basis of high NCV in some *MPZ* mutations remains unclear, and information about the influence of particular mutations on the structure and function of the *MPZ* molecule seems necessary. Our CMT2 patient group did not reveal the presence of the CMT1A duplication nor mutations in *MPZ*.

ACKNOWLEDGMENTS

The authors wish to thank E. De Vriendt, V. Van Gerwen and V. Wouters for their technical assistance, and Dr. A.V. Perepelov and Dr. G.K.Yudina for assistance in medical examinations.

REFERENCES

Bennet MVL, Barrio LC, Bargiello TA, Spray DC, Hertzberg E, Sáez JC. 1991. Gap junctions: new tools, new answers, new questions. Neuron 6:305–320.

Birouk N, LeGuern E, Maisonobe T, Rouger H, Gouider R, Tardieu S, Gugenheim M, Routon MC, Agid Y, Brice A, Bouche P. 1998. X-linked Charcot-Marie-Tooth disease with Connexin 32 (Cx32) point mutation: evidence of a primary axonal neuropathy in 48 cases. Neurology 50:1074–1082.

Blair IP, Nash J, Gordon MJ, Nicholson GA. 1996. Prevalence and origin of de novo duplications in Charcot-Marie-Tooth disease type 1A: First report of a de novo duplication with a maternal origin. Am J Hum Genet 58:472–476.

Bort S, Sevilla T, Vílchez JJ, Prieto F, Palau F. 1994. Diagnóstico y prevalencia de la duplicación del locus CMT1A en la enfermedad de Charcot-Marie-Tooth tipo 1. Med Clin (Barc) 104:648–652.

Chance PF, Bird TD, O'Connell P, Lipe HP, Lalouel J-M, Leppert M. 1990. Genetic linkage and heterogeneity in type I Charcot-Marie-Tooth disease (hereditary motor and sensory neuropathy type I). Am J Hum Genet 47:915–925.

Chance PF, Alderson MK, Leppig KA, Lensch MW, Matsunami N, Smith B, Swanson PD, Odelberg SJ, Distsche CM, Bird TD. 1993. DNA deletion associated with hereditary neuropathy with liability to pressure palsies. Cell 72:143–151.

De Jonghe P, Timmerman V, Nelis E, Martin J-J, Van Broeckhoven C. 1997. Charcot-Marie-Tooth disease and related peripheral neuropathies. J Peripher Nerv Syst 2:370–387.

De Jonghe P, Timmerman V, Van Broeckhoven C, and workshop participants. 1998. 2nd workshop of the European CMT consortium: 53rd ENMC international workshop on classification and diagnostic guidelines for Charcot-Marie-Tooth type 2 (CMT2 - HMSN II) and distal hereditary motor neuropathy (distal HMN–spinal CMT), 26–28 September 1997, Naarden, The Netherlands. Neuromusc Disord 8:426–431.

De Jonghe P, Timmerman V, Ceuterick C, Nelis E, De Vriendt E, Löfgren A, Vercruyssen A, Verellen C, Van Maldergem L, Martin J-J, Van Broeckhoven C. 1999. The Thr124Met mutation in the peripheral myelin protein zero (MPZ) gene is associated with a clinically distinct Charcot-Marie-Tooth phenotype. Brain 122:281–290.

Dyck PJ, Chance P, Lebo R, Carney JA. 1993. Hereditary motor and sensory neuropathies. In: Peripheral Neuropathy. Dyck PJ, Thomas PK, Griffin JW, et al., eds. Philadelphia: W.B. Saunders Company. p 1094–1136.

Harding AE, Thomas PK. 1980. The clinical features of hereditary motor and sensory neuropathy types I and II. Brain 103:259–280.

Hoogendijk JE, Hensels GW, Gabreëls-Festen AAWM, Gabreëls FJM, Janssen EAM, De Jonghe P, Martin J-J, Van Broeckhoven C, Valentijn LJ, Baas F, de Visser M, Bolhuis PA. 1992. De-novo mutation in hereditary motor and sensory neuropathy type 1. Lancet 339:1081–1082.

Lemke G. 1988. Unwrapping the genes for myelin. Neuron 1:535–543.

Lupski JR, Montes de Oca-Luna R, Slaugenhaupt S, Pentao L, Guzzetta V, Trask BJ, Saucedo-Cardenas O, Barker DF, Killian JM, Garcia CA, Chakravarti A, Patel PI. 1991. DNA duplication associated with Charcot-Marie-Tooth disease type 1A. Cell 66:219–239.

Lupski JR, Wise CA, Kuwano A, Pentao L, Parker J, Glaze D, Ledbetter D, Greenberg F, Patel PI. 1992. Gene dosage is a mechanism for Charcot-Marie-Tooth disease type 1A. Nature Genet 1:29–33.

Marrosu MG, Vaccargiu S, Marrosu G, Vannelli A, Cianchetti C, Muntoni F. 1998. Charcot-Marie-Tooth disease type 2 associated with mutation of the myelin protein zero gene. Neurology 50:1397–1401.

Matsunami N, Smith B, Ballard L, Lensch MW, Robertson M, Albertsen H, Hanemann CO, Müller HW, Bird TD, White R, Chance PF. 1992. Peripheral myelin protein-22 gene maps in the duplication in chromosome 17p11.2 associated with Charcot-Marie-Tooth 1A. Nat Genet 1:176–179.

Mostacciuolo ML, Schiavon F, Angelini C, Miccoli B, Piccolo F, Danieli GA. 1995. Frequency of duplication at 17p11.2 in families of North East Italy with Charcot-Marie-Tooth disease type 1 (CMT 1). Neuroepidemiology 14:49–53.

Naef R, Suter U. 1999. Impaired intracellular trafficking is a common disease mechanism of PMP22 point mutations in peripheral neuropathies. Neurobiol Dis 6:1–14.

Nelis E, Timmerman V, De Jonghe P, Vandenberghe A, Pham-Dinh D, Dautigny A, Martin J-J, Van Broeckhoven C. 1994. Rapid screening of myelin genes in CMT1 patients by SSCP analysis: identification of new mutations and polymorphisms in the P0 gene. Hum Genet 94:653–657.

Nelis E, Van Broeckhoven C. 1996a. Estimation of the mutation frequencies in Charcot-Marie-Tooth disease type 1 and hererditary neuropathy with liability to pressure palsies: a European collaborative study. Eur J Hum Genet 4:25–33.

Nelis E, Warner LE, De Vriendt E, Chance PF, Lupski JR, Van Broeckhoven C. 1996b. Comparison of single-strand conformation polymorphism and heteroduplex analysis for detection of mutations in Charcot-Marie-Tooth type 1 disease and related peripheral neuropathies. Eur J Hum Genet 4:329–333.

Nelis E, Holmberg B, Adolfsson R, Holmgren G, Van Broeckhoven C. 1997a. PMP22 Thr(118)Met: recessive CMT1 mutation or polymorphism? Nat Genet 15:13–14.

Nelis E, Simokovic S, Timmerman V, Löfgren A, Backhovens H, De Jonghe P, Martin J-J, Van Broeckhoven C. 1997b. Mutation analysis of the connexin32 (Cx32) gene in Charcot-Marie-Tooth neuropathy type 1: identification of five new mutations. Hum Mutat 9:47–52.

Nelis E, Haites N, Van Broeckhoven C. 1999. Mutations in the peripheral myelin genes and associated genes in inherited peripheral neuropathies. Hum Mutat 13:11–28.

Patel PI, Roa BB, Welcher AA, Schoener-Scott R, Trask BJ, Pentao L, Snipes GJ, Garcia CA, Francke U, Shooter EM, Lupski JR, Suter U. 1992. The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot-Marie-Tooth disease type 1A. Nat Genet 1:159–165.

Raeymaekers P, Timmerman V, Nelis E, De Jonghe P, Hoogendijk JE, Baas F, Barker DF, Martin J-J, de Visser M, Bolhuis PA, Van Broeckhoven C, HMSN Collaborative Research Group. 1991. Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1a (CMT 1a). Neuromusc Disord 1:93–97.

Reiter LT, Murakami T, Koeuth T, Pentao L, Muzny DM, Gibbs RA, Lupski JR. 1996. A recombination hotspot responsible for two inherited peripheral neuropathies is located near a *mariner* transposon-like element. Nat Genet 12:288–297.

Roa BB, Garcia CA, Pentao L, Killian JM, Trask BJ, Suter U, Snipes GJ, Ortiz-Lopez R, Shooter EM, Patel PI, Lupski JR. 1993a. Evidence for a recessive PMP22 point mutation in Charcot-Marie-Tooth disease type 1A. Nat Genet 5:189–194.

Roa BB, Garcia CA, Suter U, Kulpa DA, Wise CA, Mueller J, Welcher AA, Snipes GJ, Shooter EM, Patel PI, Lupski JR. 1993b. Charcot-Marie-Tooth disease type 1A. Association with a spontaneous point mutation in the *PMP22* gene. N Engl J Med 329:96–101.

Silander K, Meretoja P, Juvonen V, Ignatius J, Pihko H, Saarinen A, Wallden T, Herregård E, Aula P, Savontaus M-L. 1998. Spectrum of mutations in Finnish patients with Charcot-Marie-Tooth disease and related neuropathies. Hum Mutat 12:59–68.

Takashima H, Nakagawa M, Kanzaki A, Yawata Y, Horikiri T, Matsuzaki T, Suehara M, Izumo S, Osame M. 1999. Germline mosaicism of MPZ gene in Dejerine-Sottas syndrome (HMSN III) associated with hereditary stomatocytosis. Neuromusc Disord 9:232–238.

Timmerman V, Nelis E, Van Hul W, Nieuwenhuijsen BW, Chen KL, Wang S, Ben Othman K, Cullen B, Leach RJ, Hanemann CO, De Jonghe P, Raeymaekers P, van Ommen G-JB, Martin J-J, Müller HW, Vance JM, Fischbeck KH, Van Broeckhoven C. 1992. The peripheral myelin protein gene *PMP-22* is contained within the Charcot-Marie-Tooth disease type 1A duplication. Nat Genet 1:171–175.

Timmerman V, Rautenstrauss B, Reiter LT, Koeuth T, Löfgren A, Liehr T, Nelis E, Bathke KD, De Jonghe P, Grehl H, Martin J-J, Lupski JR, Van Broeckhoven C. 1997. Detection of the CMT1A/HNPP recombination "hotspot" in unrelated patients of European descent. J Med Genet 34:43–49.

Timmerman V, De Jonghe P, Ceuterick C, De Vriendt E, Löfgren A, Nelis E, Warner LE, Lupski JR, Martin J-J, Van Broeckhoven C. 1999. Novel missense mutation in the early growth response 2 gene associated with Dejerine-Sottas syndrome phenotype. Neurology 52:1827–1832.

Tobler A, Notterpek I, Naef R, Taylor V, Suter U, Shooter E. 1999. Transport of Trembler-J mutant peripheral myelin protein 22 is blocked in the intermediate compartment and affects the transport of the wild-type protein by direct interaction. J Neurosci 19:2027–2036.

Valentijn LJ, Baas F, Wolterman RA, Hoogendijk JE, van den Bosch NHA, Zorn I, Gabreëls-Festen AAWM, de Visser M, Bolhuis PA. 1992. Identical point mutations of PMP-22 in Trembler-J mouse and Charcot-Marie-Tooth disease type 1A. Nat Genet 2:288–291.

Warner LE, Roa BB, Lupski JR. 1996. Absence of PMP22 coding region mutations in CMT1A duplication patients: further evidence supporting gene dosage as a mechanism for Charcot-Marie-Tooth disease type 1A. Hum Mutat 8:362–365.

Warner LE, Mancias P, Butler IJ, McDonald CM, Keppen L, Koob G, Lupski JR. 1998. Mutations in the early growth response 2 (*EGR2*) gene are associated with hereditary myelinopathies. Nat Genet 18:382–384.