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Estimation of the Mutation Frequencies in Charcot-Marie-Tooth Disease Type 1 and Hereditary Neuropathy with Liability to Pressure Palsies: A European Collaborative Study

Eva Nelis
Christine Van Broeckhoven
and coauthors

Neurogenetics Laboratory, Flemish Institute for Biotechnology, Born-Bunge Foundation, University of Antwerp, Department of Biochemistry, Antwerp, Belgium

Coauthors: *P. De Jonghe, A. Löfgren,* Antwerp, Belgium; *A. Vandenberghe, P. Latour,* Lyon, France; *E. Le Guern, A. Brice,* Paris, France; *M.L. Mostacciuolo, F. Schiavon,* Padova, Italy; *F. Palau, S. Bort,* Valencia, Spain; *M. Upadhyaya,* Cardiff, UK; *M. Rocchi, N. Archidiacono,* Bari, Italy; *P. Mandich, E. Bellone,* Genova, Italy; *K. Silander, M.L. Savontaus,* Turku, Finland; *R. Navon, H. Goldberg-Stern,* Kfar Saba, Israel; *X. Estivill, V. Volpini,* Barcelona, Spain; *W. Friedl, K. Zerres,* Bonn, Germany; *J. Tyson, S. Malcolm,* London, UK; *B. Holmberg, G. Holmgren,* Umeå, Sweden; *E.C.M. Mariman, A.A.W.M. Gabreëls-Festen,* Nijmegen, The Netherlands; *C. Yapijakis, D. Vassilopoulos,* Athens, Greece; *C. Clark, N. Haites,* Aberdeen, UK; *P. Hilbert, L. Van Maldergem,* Loverval, Belgium; *B. Rautenstrauss, H. Grehl,* Erlangen, Germany; *R. Mountford, K. Mann,* Manchester, UK; *T. Bettecken, J.M. Burgunder,* Bern, Switzerland; *C.O. Hanemann, H.W. Müller,* Düsseldorf, Germany; *J.M. Hertz,* Aarhus, Denmark; *D.F. Schorderet, T. Küntzer,* Lausanne, Switzerland; *C. Wolf, E. Kunert,* Leipzig, Germany; *F. Muntoni,* Cagliari, Italy; *P. Emmerick-Bock,* Heidelberg, Germany; *U. Orth, A. Gal,* Lübeck, Germany

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Abstract
A European collaboration on Charcot-Marie-Tooth type 1 (CMT1) disease and hereditary neuropathy with liability to pressure palsies (HNPP) was established to estimate the duplication and deletion frequency, respectively, on chromosome 17p11.2 and to make an inventory of mutations in the myelin genes, peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ) and connexin 32 (Cx32) located on chromosomes 17p11.2, 1q21–q23 and Xq13.1, respectively. In 70.7% of 819 unrelated CMT1 patients, the 17p11.2 duplication was present. In 84.0% of 156 unrelated HNPP patients, the 17p11.2 deletion was present. In the nonduplicated CMT1 patients, several different mutations were identified in the myelin genes PMP22, MPZ and Cx32.

Introduction

The hereditary motor and sensory neuropathies (HMSNs) are a clinically heterogeneous group of peripheral neuropathies, characterized by slowly progressive weakness and atrophy of the distal limb muscles [1]. The prevalence of all types has been estimated at 1 in 10,000 [2]. HMSN type I or Charcot-Marie-Tooth disease type 1 (CMT1) is the most common form. Clinically, CMT1 is characterized by pes cavus, reduced or absent deep-tendon reflexes, and hypertrophic nerves. The age of onset of the symptoms is the first or second decade, with a considerable variation among CMT1 patients ranging from almost no symptoms to severe weakness, atrophy, and foot deformity. However, all CMT1 patients have severely reduced nerve conduction velocities (NCVs) and segmental de- and remyelination on nerve biopsy.

Positional cloning has shown that CMT1 is genetically heterogeneous with at least four distinct loci. The major autosomal dominant subtype CMT1A is linked to chromosome 17p11.2 [3, 4], a minor subtype CMT1B is linked to chromosome 1 in the region 1q22–q23 [5, 6] and a third, still unassigned subtype CMT1C is not linked to either of these loci [7]. An X-linked dominant locus was

KARGER
E-Mail karger@karger.ch
Fax + 41 61 306 12 34

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Christine Van Broeckhoven
Neurogenetics Laboratory
Born-Bunge Foundation, University of Antwerp (UIA)
Department of Biochemistry, Universiteitsplein 1
B–2610 Antwerpen (Belgium)

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mapped to chromosome Xq13 [8]. In the majority of the CMT1A patients, the disease is associated with a tandem DNA duplication of 1.5 Mb [9, 10]. This duplication is also the cause of the disease in the majority of the sporadic CMT1 cases [11, 12]. The peripheral myelin protein 22 gene (PMP22) was found to be located within the CMT1A duplication, suggesting that overexpression of this gene causes the CMT1A disease phenotype [13–16]. Point mutations in PMP22 in nonduplicated CMT1A patients confirmed the direct role of the gene in the CMT1A disease process [17–20]. The myelin protein zero gene (MPZ), the major myelin gene of the peripheral nerve, has been assigned to chromosome 1 in the region where CMT1B was previously mapped by linkage analysis studies [5]. Several distinct mutations in MPZ cosegregating with the disease identified MPZ as the CMT1B gene [21–26]. The gene encoding connexin 32 (Cx32), a gap junction protein, was found to be located in the CMTX candidate region at Xq13.1. Mutations were found in 24 out of 27 X-linked CMT1 families [27–30]. The expression of Cx32 in the peripheral nerve was not known before [27].

Hereditary neuropathy with liability to pressure palsies (HNPP), also called tomaculous neuropathy, is characterized by periodic episodes of numbness and palsies that follow relatively minor compression or trauma to the peripheral nerves. Electrophysiology may reveal reduced motor and sensory NCVs in clinically affected patients and asymptomatic carriers. On nerve biopsy, tomaculae or sausage-like structures are present. HNPP is inherited as an autosomal dominant trait [31]. The disease is usually associated with a deletion in chromosome 17p11.2 [32]. In the vast majority of cases, the deletion comprises all markers that are duplicated in CMT1A. Furthermore, the duplication and the deletion arise from the same unequal crossing-over event at the CMT1A-REP site, a repeat sequence flanking the CMT1A region [33, 34]. Therefore, the CMT1A duplication and the HNPP deletion are reciprocal mutations. A decreased dosage of PMP22 is a possible cause of HNPP. This hypothesis is supported by the fact that a nondeleted HNPP patient was identified who carried a 2-bp deletion in PMP22 causing early termination of transcription [35].

To estimate the frequencies of the CMT1A duplication and the HNPP deletion in Europe, and to make an inventory of the different mutations in PMP22, MPZ, and Cx32, the data of several European research centers were pooled. A first European Neuromuscular Center (ENMC)-sponsored workshop on hereditary motor and sensory neuropathies was organized in Baarn, The Netherlands, in May 1991 [36]. Here it was decided to establish diagnostic criteria for HMSN type I, and to define the requirements for individuals to be included in a linkage analysis [36]. Briefly, these criteria are: (1) slowly progressive symmetrical muscle wasting and weakness, predominantly of the distal part of the lower limbs; (2) severely decreased motor median NCVs ($\leq$30 m/s), absence or marked decrease of sensory nerve action potentials (SNAPs) in the lower limbs and/or a sensory nerve biopsy consistent with a diagnosis of demyelinating neuropathy, and (3) pedigree consistent with autosomal dominant inheritance. The low cutoff NCV value of 30 m/s was used since such low NCV values have been observed only in CMT1 patients, ensuring that only families with a CMT1 phenotype would be ascertained in the linkage analysis studies. At this meeting, a European CMT consortium group was also formed. A second ENMC-sponsored workshop was organized in Den Dolder, The Netherlands, in December 1992 [37]. Members of the CMT consortium group involved in DNA diagnosis of CMT patients were sent a questionnaire to assess the frequency and the size of the 17p11.2 duplication in their patients with a CMT1 phenotype, to determine the frequency of new mutation cases in sporadic CMT1 patients, and to evaluate possible screening methods for the CMT1 duplication. Only data obtained on patients with a well-defined phenotype were included. Selection criteria for the familial cases were as defined at the first workshop. The clinical and electrophysiological criteria were the same for the sporadic CMT1 patients. In addition, both parents had to be clinically and electrophysiologically normal and analyzed for the duplication, and paternity of the patients had to be confirmed. Data from 20 laboratories in 11 countries were retained in the final analysis, resulting in a duplication frequency of 84.6% of the familial CMT1 cases (total of 273 families), and a de novo duplication frequency of 93% (total of 28 patients) [37]. In 9 of the de novo duplications, the parental origin could be deduced: all 9 were paternally derived [12].

At the 26th annual meeting of the European Society of Human Genetics in Paris, France, in June 1994, a meeting of the European CMT consortium group was organized to update the data on the duplication frequency in CMT1 patients, to make a first inventory of the type and frequency of mutations in PMP22, MPZ, and Cx32, and to estimate the deletion frequency in patients with HNPP. All members of the CMT consortium group were asked to complete a questionnaire concerning duplication, deletion, and mutation screening of CMT1 and HNPP patients. The CMT1 patients had to fulfil all clinical and electrophysiological criteria as defined at the first work-

shop. However, since we wanted to ascertain all CMT1 patients, a cutoff value of 38 m/s for the motor median nerve was used, as proposed by Harding and Thomas [38] on the basis of motor median NCV measurements in 170 CMT patients [38]. Furthermore, no restrictions were made with respect to patients' family history, since we also wanted to assess the duplication/deletion frequencies in clinically isolated and genetically sporadic cases. For HNPP patients, no consensus diagnosis was available and thus each center used their own diagnostic criteria. We received the results from 28 centers, the data were compiled and are discussed in this paper.

Methods

Duplication and Deletion Screening

The most common technique used in the different participating centers to detect the duplication was the scoring of three alleles or dosage differences on Southern blot hybridization: intensities of heterozygous RFLP alleles were visually compared one versus the other. Subclones [39] of the following markers, free of repetitive sequences, were provided by the European CMT consortium group: pVAW409R1, pVAW409R3, pVAW412R3, or pEW401. One or more of these markers, or the PMP22 probe p132G8R1 [13], were used in the analysis. The scoring of triple alleles was also done using the microsatellite markers RM11-GT and Mfd41 [10, 40]. In a few laboratories, the duplication was identified by quantification of alleles of fluorescent-labeled microsatellite markers RM11-GT, Mfd41, AFM191xh12, AFM317yg1, and AFM200yb12, using ABI GENESCAN 672 software (Applied Biosystems, Foster City, Calif., USA) [41, 42]. A few centers used additional techniques: fluorescence in situ hybridization (FISH) or pulsed-field gel electrophoresis (PFGE). FISH analysis detects three spots of a marker located within the CMT1A region compared to two spots of a marker located outside the duplication [10]. With PFGE, a novel junction fragment of 500 kb can be detected in DNA digested with different rare cutter restriction enzymes such as *Sac*II, *Fsp*I, or *Asc*I, and hybridized with the marker pVAW409R3 or probes from the CMT1A-REP region [10, 15, 33, 39].

Similar methods were used to detect the deletion in HNPP patients: scoring of loss of alleles on Southern blot hybridization or microsatellite markers and FISH analysis. In this study, PFGE was not used in the diagnosis of HNPP, although it is now possible to detect junction fragments in HNPP patients with markers from the CMT1A-REP region [19, 34, 43, 44].

Mutation Analysis

The techniques used for mutation screening in CMT1 and HNPP were single-stranded conformation polymorphism (SSCP) analysis, heteroduplex analysis or direct sequencing of the coding regions of the myelin genes PMP22, MPZ, or Cx32. SSCP and heteroduplex analysis do not detect all the mutations. The sensitivity of SSCP and heteroduplex analysis is estimated at 80% [45]. Direct sequencing should reveal all mutations localized in the coding region of the genes. However, since this technique is very time-consuming and expensive, it was not routinely used in the DNA diagnostic laboratories.

Results

CMT1A Duplication Frequency

A total of 881 unrelated CMT1 patients were tested for the presence of the CMT1A duplication by one or more of the techniques mentioned above. 819 patients were informative for the duplication analysis, i.e. 579 patients were duplicated and 240 patients were not. The remaining 62 patients were uninformative. These data resulted in a duplication frequency of 70.7% (table 1). Part of the duplication screening results of the individual participating centers have been published elsewhere [9, 39, 46–54]. Only 6 out of 579 (1.0%) patients were found to have a smaller duplication. One of these cases was described by Palau et al. [12]. The CMT1A duplication frequency varied significantly between the different centers. The highest frequency was 100.0%, the lowest 34.3% (table 1). In principle, the duplication frequencies could be biased towards higher frequencies if some of the centers would have included related CMT1 patients. However, to the best of our knowledge, the patients under study were unrelated, since the different centers contributed only 1 patient sample per CMT1 family. Also, the differences in frequency did not seem to reflect a different ethnic origin, since high and low frequencies occurred in the same country. However, in more isolated populations, like northern Sweden, the low duplication frequency (37.5%) could be caused by a relatively higher frequency of recessive CMT1 cases [51].

The duplication frequency of the familial cases, i.e. cases with at least one other known CMT1 patient in the family, was 75.9% (477/628). If we selected only the proven autosomal dominant cases, the duplication frequency was 85.2% (table 1). When we also included the dominant cases, i.e. cases belonging to families without male-to-male transmission, the duplication frequency decreased to 78.4%, because in the latter group, the duplication frequency was only 63.1% (111/176). An explanation for this low duplication frequency could be that this group of dominant cases also comprises patients with X-linked CMT1.

In clinically isolated cases, i.e. cases which had no family history of the disease or cases for which data on family history were not available, it was 53.4% (102/191). If only genetically sporadic cases were considered, i.e. cases with both parents clinically and electrophysiologically normal, analyzed for the duplication and paternity confirmed, a de novo duplication was observed in 76.5% of the cases (table 1). Some of the individual participating centers have published their data elsewhere [11, 12, 55]. The de novo duplication cases represent 6.7% of the total number of duplicated CMT1 patients (39/579).

Table 1. Patients tested for the CMT1A duplication and the HNPP deletion

Center	Duplication frequency in CMT1, %			Deletion frequency in HNPP, %		
	total	AD	de novo	total	AD	de novo
Turku, Finland	84.0 (25)	100.0 (5)	50.0 (2)	100.0 (20)	100.0 (6)	–
Umeå, Sweden	37.5 (24)	100.0 (9)	–			
Aarhus, Denmark	54.5 (11)	62.5 (8)	100.0 (1)			
Aberdeen, UK	60.0 (15)	100.0 (2)	–	100.0 (1)	–	–
Manchester, UK	77.8 (18)	85.7 (14)	–			
London, UK				69.2 (26)	66.7 (21)	100.0 (1)
Cardiff, UK	75.8 (33)	75.0 (32)	100.0 (1)			
Nijmegen, The Netherlands				70.8 (24)	76.2 (21)	100.0 (1)
Loverval, Belgium	44.4 (18)	57.1 (7)	–	100.0 (1)	100.0 (1)	–
Antwerp, Belgium	69.7 (66)	73.7 (19)	83.3 (6)	100.0 (11)	100.0 (4)	100.0 (1)
Heidelberg, Germany	100.0 (3)	100.0 (3)	–			
Bonn, Germany	81.5 (27)	50.0 (2)	–	50.0 (2)	–	–
Düsseldorf, Germany	81.8 (11)	–	–	100.0 (2)	–	–
Leipzig, Germany	100.0 (9)	–	–			
Erlangen, Germany	61.1 (18)	50.0 (4)	0.0 (1)	100.0 (1)	100.0 (1)	–
Lyon, France	72.9 (155)	98.1 (104)	100.0 (11)	69.6 (23)	100.0 (15)	100.0 (1)
Paris, France	76.1 (67)	89.8 (49)	100.0 (3)	100.0 (13)	100.0 (13)	–
Bern, Switzerland	76.5 (17)	81.8 (11)	–			
Lausanne, Switzerland	100.0 (10)	100.0 (1)	–	100.0 (1)	–	–
Genova, Italy	97.1 (35)	96.7 (30)	100.0 (5)	100.0 (11)	100.0 (6)	–
Padova, Italy	73.4 (64)	83.3 (24)	100.0 (5)	100.0 (1)	100.0 (1)	–
Cagliari, Italy	62.5 (8)	50.0 (6)	–			
Bari, Italy	65.9 (41)	79.2 (24)	11.1 (9)	87.5 (8)	100.0 (3)	66.7 (3)
Barcelona, Spain	34.3 (35)	28.6 (7)	–			
Valencia, Spain	67.3 (49)	100.0 (12)	85.7 (7)	87.5 (8)	100.0 (3)	–
Athens, Greece	40.0 (16)	30.0 (10)	–	100.0 (2)	100.0 (2)	–
Kfar Saba, Israel	85.0 (40)	93.3 (15)	–			
Total	70.7 (819)	85.2 (398)	76.5 (51)	84.0 (156)	87.6 (97)	85.7 (7)

Values in parentheses are the total number of patients informative in the duplication/deletion screening. AD = autosomal dominant.

HNPP Deletion Frequency

162 HNPP patients were screened for the deletion. 156 patients were informative for the deletion analysis, i.e., 131 patients had the deletion, 25 were not deleted, while the remaining 6 patients were not informative. These data resulted in an overall deletion frequency of 84.0% in the HNPP patients. Deletion screening results of some individual participating centers have been published [44, 56–59]. 5 out of 131 (3.8%) of the deleted HNPP patients had a smaller deletion; 1 of these patients is described by Chapon et al. [submitted].

The deletion frequency in the familial cases was 86.1% (105/122), that in the proven autosomal dominant cases was 87.6% (table 1), and in the dominant cases 89.5% (17/19). These comparable deletion frequencies suggest that an X-linked dominant form of HNPP is rather unlikely.

The deletion frequency in the clinically isolated cases was 76.5% (26/34). In 85.7% of the genetically sporadic cases, a de novo deletion was present (table 1). The de novo deletion cases represent 4.6% (6/131) of the total number of deleted HNPP patients.

Mutations in Myelin Genes

Mutation screening of the PMP22, MPZ, and Cx32 genes was performed in 13 of the participating centers in 40.8% (98), 44.6% (107) and 15.0% (36), respectively, of the nonduplicated CMT1 patients. In 3 out of 98 (4.1%) patients, a sequence variation in PMP22 was detected by SSCP analysis. In one of these patients, the mutation has

Table 2. CMT1 and HNPP mutations

Disease	Gene	Exon/codon	Nucleotide change	Type	Effect on coding sequence	Center	Reference
CMT1	PMP22	IVS3+1	TGgt→TGat	5′ splice site	–	Antwerp, Belgium	[20]
		4/147	CTG→CGG	missense	Leu→Arg	Kfar Saba, Israel	[63]
	MPZ	2/63	TCC→TTC	missense	Ser→Phe	Lyon, France	[64]
		2/78[a]	TCG→TTG	missense	Ser→Leu	Antwerp, Belgium	[26]
		2/78	TCG→TTG	missense	Ser→Leu	Lyon, France	[65]
		2/81	CAC→CGC	missense	His→Arg	Cardiff, UK	
		3/86–89	8 bp replaced by 5 bp			Turku, Finland	[66]
		3/101	TGG→TGC	missense	Trp→Cys	Lyon, France	[65]
		3/122	AAT→AGT	missense	Asn→Ser	Lyon, France	[67]
		3/134	GAC→AAC	missense	Asp→Glu	Antwerp, Belgium	[25]
		3/134	GAC→GAA	missense	Asp→Asn	Antwerp, Belgium	[26]
		4/154[a]	TAC→TAA	nonsense	Tyr→stop	Antwerp, Belgium	[26]
		4/163	GGG→AGG	missense	Gly→Arg	Antwerp, Belgium	
		4/167	GGG→AGG	missense	Gly→Arg	Cardiff, UK	
		4/181	TAC→TAG	nonsense	Tyr→stop	Antwerp, Belgium	[26]
		6/221	insertion of GC	frameshift	–	Erlangen, Germany	[68]
		6/223	deletion of 4 bp	frameshift	Ser233del	Genova, Italy	[69]
	Cx32	2/15[b]	CGG→TGG	missense	Arg→Trp	Lübeck, Germany	
		2/15[a]	CGG→TGG	missense	Arg→Trp	Umeå, Sweden	
		2/15[b]	CGG→CAG	missense	Arg→Gln	Aberdeen, UK	[28]
		2/22	CGA→CAA	missense	Arg→Gln	Aberdeen, UK	
		2/25[a] (×2)	CTC→TTC	missense	Leu→Phe	Antwerp, Belgium	[70]
		2/26	TCG→TTG	missense	Ser→Leu	Antwerp, Belgium	[70]
		2/26[a]	TCG→TTG	missense	Ser→Leu	Antwerp, Belgium	[70]
		2/38[b]	GTG→ATG	missense	Val→Met	Lübeck, Germany	[30]
		2/40	CGA→GTA	missense	Ala→Val	Aberdeen, UK	
		2/56	CTC→TTC	missense	Leu→Phe	Aberdeen, UK	
		2/60[b]	TGC→TTC	missense	Cys→Phe	Aberdeen, UK	[28]
		2/72–73	deletion of C	frameshift	early stop	Aberdeen, UK	[28]
		2/72–73[b]	deletion of C	frameshift	early stop	Aberdeen, UK	[28]
		2/87	CCA→GCA	missense	Pro→Ala	Antwerp, Belgium	[70]
		2/87[a]	CCA→GCA	missense	Pro→Ala	Antwerp, Belgium	[70]
		2/93[a]	ATG→GTG	missense	Met→Val	Aberdeen, UK	
		2/139[b]	GTG→ATG	missense	Val→Met	London, UK	
		2/142[b]	CGG→TGG	missense	Arg→Trp	Lübeck, Germany	
		2/208	GAG→AAG	missense	Glu→Lys	Aberdeen, UK	[28]
		2/215[b]	CGG→TGG	missense	Arg→Trp	Aberdeen, UK	[28]
		2/220	CGA→TGA	nonsense	Arg→stop	Aberdeen, UK	[28]
		2/238	CGC→CAC	missense	Arg→His	Antwerp, Belgium	[70]
		2/281	TCG→TAG	nonsense	Ser→stop	Antwerp, Belgium	[70]
		2/?[b]	deletion of 18 bp			London, UK	
		2/?[b]	deletion of 11 bp			London, UK	
HNPP	PMP22	IVS1+1	GCgt→GCtt	5′ splice site	–	Valencia, Spain	

IVS = intervening sequence; × 2 = mutation observed in 2 unrelated CMT1 patients.

[a] Patients not fulfilling the criteria defined by the European CMT consortium group or patients not informative for the duplication.

[b] Patients from X-linked pedigrees.

been identified by sequencing (table 2). A PMP22 mutation was also identified by sequencing in a CMT1 patient with no altered SSCP pattern [20]. In MPZ, 14 sequence variations were observed by SSCP or heteroduplex analysis in 107 patients (13.1%). 13 MPZ mutations have already been identified by sequencing (table 2). In addition, 2 MPZ mutations were found in patients not included in table 1, since they did not fulfil the inclusion criteria (no NCV values were available, table 2). Screening of the Cx32 gene by SSCP analysis and sequencing revealed 10 mutations in 36 patients (27.8%, table 2). A further 17 mutations in Cx32 were identified, 6 in patients not fulfilling all inclusion criteria of this study, 10 in proven X-linked families, and 1 in a patient not informative for the CMT1A duplication (table 2).

Eight out of the 25 (32.0%) nondeleted HNPP cases were screened for mutations in the PMP22 and MPZ genes. SSCP analysis revealed one sequence variation in exon 1 of PMP22 segregating with the disease in an autosomal dominant HNPP family. Sequencing identified a splice donor site mutation [Bort, pers. commun.].

Discussion

In this European collaboration study on CMT1 and HNPP mutation frequencies, 70.7% of 819 unrelated CMT1 patients had the CMT1A duplication. A similar duplication frequency of 68% was found in a smaller study of 63 unrelated CMT1 patients from the USA [60]. The duplication frequencies in the familial cases was 75.9%, in the clinically isolated cases 53.4%. The duplication frequency in the autosomal cases was 85.2%, that in genetically sporadic cases 76.5%. In 84.0% of the 156 unrelated HNPP patients, the 17p11.2 deletion was present. The nonavailability of strict diagnostic criteria for HNPP hampered the evaluation of the results from the different centers. Consequently, the frequencies varied widely among the different centers. The deletion frequency in the familial cases was 86.1%, in the clinically isolated cases 76.5%. The deletion frequency in the autosomal cases was 87.6%, that in genetically sporadic cases 85.7%. Only 1.0% of the duplicated CMT1 patients had a smaller duplication, while 3.8% of the HNPP patients had a smaller deletion. These numbers are possibly an underestimation of the real number of smaller duplications/deletions, since not all smaller duplications/deletions may be recognized if not all markers of the CMT1A region were analyzed. However, a recent report demonstrates that the same 500-kb junction fragment was found in 512 CMT1A duplication patients [61], confirming that a smaller duplication/deletion seems to be a very rare event in CMT1A and HNPP disease. The fact that the duplication and the deletion comprised exactly the same region in the vast majority of the CMT1 and HNPP patients supported the hypothesis that the duplication/deletion arises from an unequal crossing-over event at the CMT1A-REP site, the repeat sequence flanking the CMT1A/HNPP region [33, 34]. The high frequency of de novo duplication/deletion cases in CMT1A and HNPP suggests a relative high mutation rate at 17p11.2. The high duplication/deletion frequencies in CMT1/HNPP patients indicate that the duplication/deletion screening is an important molecular genetic tool allowing a molecular diagnosis in the majority of these patients.

In 7.0% (62/881) of the CMT1 patients and 3.7% (6/162) of the HNPP patients, the duplication/deletion screening was not informative. The informativeness of the duplication/deletion screening depends on the detection method. The FISH and PFGE methods are 100% informative, but are not routinely used in most centers. In the Southern blot hybridization and microsatellite analysis, the informativeness depends on the heterozygosity of the alleles. A method to partially solve this problem is to use a nonduplicated reference marker in the Southern blot hybridization to compare dosages in homozygous patients. In general, it is difficult to quantify PCR products, particularly when radioactive labeling is used. Therefore, in microsatellite analysis, the presence of the duplication is most reliably diagnosed if three alleles are present [60]. However, automated analysis of fluorescence-labeled microsatellite markers allows the identification of the duplication by quantification of the alleles [41, 42].

Mutation screening of the CMT1 myelin genes in nonduplicated CMT1 patients is not yet a routine technique in most laboratories. Only part of the nonduplicated CMT1 cases were screened by SSCP analysis and/or direct sequencing. Therefore an exact estimation of the frequency of CMT1 mutations cannot be made. Based on the total number of mutations in the CMT1 genes reported here and by others, it is clear that mutations in Cx32 are more frequent than MPZ mutations, and that they are both much more frequent than PMP22 mutations. Therefore, for diagnostic purposes, nonduplicated CMT1 patients should be screened first for mutations in Cx32 (unless male-to-male transmission occurred in the family) and then in MPZ, before screening for PMP22 mutations. Eight of the 25 nondeleted HNPP patients were tested for mutations in PMP22 and/or MPZ. In 1 patient, a splice donor site mutation in PMP22 was iden-

tified [Bort, pers. commun.]. Since not all HNPP patients had a deletion or a mutation in PMP22 and since linkage analysis in some of the nondeleted families excluded a large part of chromosome 17p [62], the data confirmed that HNPP is genetically heterogeneous with a locus on 17p11.2 and at least one other locus still to be identified.

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References

1 Dyck PJ, Chance P, Lebo R, Carney JA: Hereditary motor and sensory neuropathies; in Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF (eds): Peripheral Neuropathy. Philadelphia, Saunders, 1993, pp 1094–1136.

2 Emery AEH: Population frequencies of inherited neuromuscular diseases – A world survey. Neuromuse Disord 1991;1:19–29.

3 Vance JM, Nicholson GA, Yamaoka LH, Stajich J, Stewart JS, Speer MC, Hung W, Roses AD, Barker D, Pericak-Vance MA: Linkage of Charcot-Marie-Tooth neuropathy type 1a to chromosome 17. Exp Neurol 1989;104:186–189.

4 Raeymaekers P, Timmerman V, De Jonghe P, Swerts L, Gheuens J, Martin J, Muylle L, De Winter G, Vandenberghe A, Van Broeckhoven C: Localization of the mutation in an extended family with Charcot-Marie-Tooth neuropathy (HMSN I). Am J Hum Genet 1989;45:953–958.

5 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.

6 Lebo R, Chance PF, Dyck PJ, Redila-Flores M, Lynch E, Golbus M, Bird T, King M, Anderson LA, Hall J, Wiegant J, Jiang Z, Dazin PF, Punnett HH, Schonberg SA, Moore K, Shull MM, Gendler S, Hurko O, Lovelace RE, Latov N, Trofatter J, Conneally PM: Chromosome 1 Charcot-Marie-Tooth disease (CMT1B) locus in the Fc gamma receptor gene region. Hum Genet 1991;88:1–12.

7 Chance PF, Matsunami N, Lensch MW, Smith B, Bird TD: Analysis of the DNA duplication 17p11.2 in Charcot-Marie-Tooth neuropathy type 1 pedigrees: Additional evidence for a third autosomal CMT1 locus. Neurology 1992; 42:2037–2041.

8 Gal A, Mücke J, Theile H, Wieacker PF, Ropers HH, Wienker TF: X-linked dominant Charcot-Marie-Tooth disease: Suggestion of linkage with a cloned DNA sequence from the proximal Xq. Hum Genet 1985;70:38–42.

9 Raeymaekers P, Timmerman V, Nelis E, De Jonghe P, Hoogendijk JE, Baas F, Barker DF, Martin J, de Visser M, Bolhuis PA, Van Broeckhoven C, HMSN Collaborative Research Group: Charcot-Marie-Tooth neuropathy type 1a (CMT1a) is most likely caused by a duplication in chromosome 17p11.2. Neuromuse Disord 1991;1:93–97.

10 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: DNA duplication associated with Charcot-Marie-Tooth disease type 1A. Cell 1991;66:219–239.

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

12 Palau F, Löfgren A, De Jonghe P, Bort S, Nelis E, Sevilla T, Martin J, Vílchez J, Prieto F, Van Broeckhoven C: Origin of the de novo duplication in Charcot-Marie-Tooth disease type 1A: Unequal nonsister chromatid exchange during spermatogenesis. Hum Mol Genet 1993;2: 2031–2035.

13 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: The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot-Marie-Tooth disease type 1A. Nat Genet 1992;1:159–165.

14 Valentijn LJ, Bolhuis PA, Zorn I, Hoogendijk JE, van den Bosch N, Hensels GW, Stanton V Jr, Housman DE, Fischbeck KH, Ross DA, Nicholson GA, Meershoek EJ, Dauwerse HG, van Ommen GB, Baas F: The peripheral myelin gene PMP-22/GAS-3 is duplicated in Charcot-Marie-Tooth disease type 1A. Nat Genet 1992;1:166–170.

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

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

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

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

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

20 Nelis E, Timmerman V, De Jonghe P, Van Broeckhoven C: Identification of a 5′ splice site mutation in the PMP-22 gene in autosomal dominant Charcot-Marie-Tooth disease type 1. Hum Mol Genet 1994;3:515–516.

21 Hayasaka K, Himoro M, Sato W, Takada G, Uyemura K, Shimizu N, Bird T, Conneally PM, Chance PF: Charcot-Marie-Tooth neuropathy type 1B is associated with mutations of the myelin P0 gene. Nat Genet 1993;5:31–34.

22 Hayasaka K, Ohnishi A, Takada G, Fukushima Y, Murai Y: Mutation of the myelin P0 gene in Charcot-Marie-Tooth neuropathy type 1. Biochem Biophys Res Commun 1993;194:1317–1322.

23 Hayasaka K, Takada G, Ionasescu VV: Mutation of the myelin P0 gene in Charcot-Marie-Tooth neuropathy type 1B. Hum Mol Genet 1993;2:1369–1372.

24 Himoro M, Yoshikawa H, Matsui T, Mitsui Y, Takahashi M, Kaido M, Nishimura T, Sawaishi Y, Takada G, Hayasaka K: New mutation of the myelin P0 gene in a pedigree of Charcot-Marie-Tooth neuropathy type 1. Biochem Mol Biol Int 1993;31:169–173.

25 Nelis E, Timmerman V, De Jonghe P, Muylle L, Martin J, Van Broeckhoven C: Linkage and mutation analysis in an extended family with Charcot-Marie-Tooth disease type 1B. J Med Genet 1994;31:811–815.
26 Nelis E, Timmerman V, De Jonghe P, Vandenberghe A, Pham-Dinh D, Dautigny A, Martin J, Van Broeckhoven C: Rapid screening of myelin genes in CMT1 patients by SSCP analysis: Identification of new mutations and polymorphisms in the P0 gene. Hum Genet 1994;94: 653–657.
27 Bergoffen J, Scherer SS, Wang S, Oronzi Scott M, Bone LJ, Paul DL, Chen K, Lensch MW, Chance PF, Fischbeck KH: Connexin mutations in X-linked Charcot-Marie-Tooth disease. Science 1993;262:2039–2042.
28 Fairweather N, Bell C, Cochrane S, Chelly L, Wang S, Mostacciuolo ML, Monaco MP, Haites NE: Mutations in the connexin 32 gene in X-linked dominant Charcot-Marie-Tooth disease (CMTX1). Hum Mol Genet 1994;3: 29–31.
29 Ionasescu V, Searby C, Ionasescu R: Point mutations of the connexin32 (GJB1) gene in X-linked dominant Charcot-Marie-Tooth neuropathy. Hum Mol Genet 1994;3:355–358.
30 Orth U, Fairweather N, Exler MC, Schwinger E, Gal A: X-linked dominant Charcot-Marie-Tooth neuropathy: Valine-38-methionine substitution of connexin32. Hum Mol Genet 1994; 3:1699–1700.
31 Windebank AJ: Inherited recurrent focal neuropathies; in Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF (eds): Peripheral Neuropathy. Philadelphia, Saunders, 1993, pp 1137–1148.
32 Chance PF, Alderson MK, Leppig KA, Lensch MW, Matsunami N, Smith B, Swanson PD, Odelberg SJ, Distsche CM, Bird TD: DNA deletion associated with hereditary neuropathy with liability to pressure palsies. Cell 1993;72: 143–151.
33 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. Nat Genet 1992;2:292–300.
34 Chance PF, Abbas N, Lensch MW, Pentao L, Roa BB, Patel PI, Lupski JR: Two autosomal dominant neuropathies result from reciprocal DNA duplication/deletion of a region on chromosome 17. Hum Mol Genet 1994;3:223–228.
35 Nicholson GA, Valentijn LJ, Cherryson AK, Kennerson ML, Bragg TL, De Kroon RM, Ross DA, Pollard JD, McLeod JD, Bolhuis PA, Baas F: A frame shift mutation in the PMP22 gene in hereditary neuropathy with liability to pressure palsies. Nat Genet 1994;6:263–266.
36 de Visser M: Diagnostic criteria for autosomal dominant hereditary motor and sensory neuropathy type 1a. Neuromusc Disord 1993;3: 77–79.
37 Van Broeckhoven C: Report of the ENMC sponsored workshop on hereditary motor and sensory neuropathies; in Anonymous (ed): ENMC Annual Report 1992. 1993, pp 36–37.
38 Harding AE, Thomas PK: The clinical features of hereditary motor and sensory neuropathy types I and II. Brain 1980;103:259–280.
39 Raeymaekers P, Timmerman V, Nelis E, Van Hul W, De Jonghe P, Martin J, Van Broeckhoven C, HMSN Collaborative Research Group: 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.
40 Chevillard C, Le Paslier D, Passage D, Ougen P, Billault A, Boyer S, Mazan S, Bachellerie JP, Vignal A, Cohen D, Fontes M: Relationship between Charcot-Marie-Tooth 1A and Smith-Magenis regions: snU3 may be a candidate gene for the Smith-Magenis syndrome. Hum Mol Genet 1993;2:1235–1243.
41 Navon R, Timmerman V, Löfgren A, Liang P, Nelis E, Zeitune M, Van Broeckhoven C: Prenatal diagnosis of Charcot-Marie-Tooth disease type 1A (CMT1A) using molecular genetic techniques. Prenatal diagnosis 1995;15:633–640.
42 Mann K, Mountford R: Molecular genetic analysis of Charcot-Marie-Tooth disease type I (abstract). Med Genet 1995;2:184.
43 Lorenzetti D, Parcyson D, Sghirlanzoni A, Roa BB, Abbas NE, Pandolfo M, Di Donato S, Lupski JR: A 1.5 Mb deletion in 17p11.2–p12 is frequently observed in Italian families with hereditary neuropathy with liability to pressure palsies. Am J Hum Genet 1995;56:91–98.
44 Timmerman V, Löfgren A, Le Guern E, Liang P, De Jonghe P, Martin J, Verhalle D, Robberecht W, Gouider R, Brice A, Van Broeckhoven C: Molecular genetic analysis of the 17p11.2 region in patients with hereditary neuropathy with liability to pressure palsies (HNPP). Hum Genet 1996;97:26–34.
45 Grompe M: The rapid detection of unknown mutations in nucleic acids. Nat Genet 1993;5: 111–117.
46 Bellone E, Mandich P, Mancardi GL, Schenone A, Uccelli A, Abbruzzese M, Sghirlanzoni A, Pareyson D, Ajmar F: Charcot-Marie-Tooth (CMT) 1a duplication at 17p11.2 in Italian families. J Med Genet 1992;29:492–493.
47 Brice A, Ravisé N, Stevanin G, Gugenheim M, Bouche P, Penet C, Agid Y, French CMT Research Group: Duplication within chromosome 17p11.2 in 12 families of French ancestry with Charcot-Marie-Tooth disease type 1a. J Med Genet 1992;29:807–812.
48 Hallam PJ, Harding AE, Berciano J, Barker DF, Malcolm S: Duplication of part of chromosome 17 is commonly associated with hereditary motor and sensory neuropathy type 1 (Charcot-Marie-Tooth disease type 1). Ann Neurol 1992;31:570–572.
49 MacMillan JC, Upadhyaya M, Harper PS: Charcot-Marie-Tooth disease type 1a (CMT1a): Evidence for trisomy of the region p11.2 of chromosome 17 in south Wales families. J Med Genet 1992;29:12–13.
50 Müller E, Mostacciuolo ML, Micaglio G, Angelini C, Danieli GA: Further evidence of a duplication in 17p11.2 in families with recurrence of HMSN Ia (Charcot-Marie-Tooth neuropathy type Ia). Hum Genet 1992;90:231–234.
51 Holmberg BH, Holmgren G, Nelis E, Van Broeckhoven C, Westerberg B: Charcot-Marie-Tooth disease in northern Sweden: Pedigree analysis and the presence of the duplication within chromosome 17p11.2. J Med Genet 1994;31:435–441.
52 Mostacciuolo ML, Schiavon F, Angelini C, Miccoli B, Piccolo F, Danieli GA: Frequency of duplication at 17p11.2 in families of north east Italy with Charcot-Marie-Tooth disease type 1 (CMT 1). Neuroepidemiology 1995;14:49–53.
53 Schiavon F, Mostacciuolo ML, Saad F, Merlini L, Siciliano G, Angelini C, Danieli GA: Non-radioactive detection of 17p11.2 duplication in CMT1A: A study on 78 patients. J Med Genet 1994;31:880–883.
54 Bort S, Sevilla T, Vílchez JJ, Prieto F, Palau F: Diagnóstico y prevalencia de la duplicación del locus CMT1A en la enfermedad de Charcot-Marie-Tooth tipo 1. Med Clin (Barc) 1994;104: 648–652.
55 Hertz JM, Borglum AD, Brandt CA, Flint T, Bisgaard C: Charcot-Marie-Tooth disease type 1A: The parental origin of a de novo 17p11.2–p12 duplication in a sporadic case. Clin Genet 1994;46:291–294.
56 Le Guern E, Sturz F, Gugenheim M, Gouider R, Bonnebouche C, Ravisé N, Gonnaud PM, Tardieu S, Bouche P, Chazot G, Agid Y, Vandenberghe A, Brice A: Detection of deletion within 17p11.2 in 7 French families with hereditary neuropathy with liability to pressure palsies (HNPP). Cytogenet Cell Genet 1994; 65:261–264.
57 Silander K, Halonen P, Sara R, Kalimo H, Falck B, Savontaus M: DNA analysis in Finnish patients with hereditary neuropathy with liability to pressure palsies (HNPP). J Neurol Neurosurg Psychiatry 1994;57:1260–1262.
58 Verhalle D, Löfgren A, Nelis E, Dehaene I, Theys P, Lammens M, Dom R, Van Broeckhoven C, Robberecht W: Deletion in the CMT1A locus on chromosome 17p11.2 in hereditary neuropathy with liability to pressure palsies. Ann Neurol 1994;35:704–708.
59 Mariman ECM, Gabreëls-Festen AAWM, van Beersum SEC, Valentijn LJ, Baas F, Bolhuis PA, Jongen PJH, Ropers HH, Gabreëls FJM: Prevalence of the 1.5-Mb 17p deletion in families with hereditary neuropathy with liability to pressure palsies. Ann Neurol 1994;36:650–655.
60 Wise CA, Garcia CA, Davis SN, Heju Z, Pentao L, Patel PI, Lupski JR: Molecular analyses of unrelated Charcot-Marie-Tooth (CMT) disease patients suggest a high frequency of the CMT1A duplication. Am J Hum Genet 1993; 53:853–863.
61 Roa BB, Ananth U, Garcia CA, Lupski JR: Molecular diagnosis of CMT1A and HNPP. Labmed Int 1995;12:22–24.

62 Mariman ECM, Gabreëls-Festen AAWM, van Beersum SEC, Jongen PJH, van de Looij E, Baas F, Bolhuis PA, Ropers HH, Gabreëls FJM: Evidence for genetic heterogeneity underlying hereditary neuropathy with liability to pressure palsies. Hum Genet 1994;93:151–156.
63 Navon R, Seifried B, Gal-On NS, Sadeh M: A new point mutation in the PMP-22 gene associated with a de novo severe CMT1A phenotype. Hum Genet, in press.
64 Blanquet-Grossard F, Pham-Dinh D, Dautigny A, Latour P, Bonnebouche C, Corbillon E, Chazot G, Vandenberghe A: Charcot-Marie-Tooth type 1B neuropathy: Third mutation at the serine 63 codon in the major peripheral myelin glycoprotein P0 gene. Clin Genet, in press.
65 Latour P, Blanquet F, Nelis E, Bonnebouche C, Chapon F, Diraison P, Ollagnon E, Dautigny A, Pham-Dinh D, Chazot G, Boucherat M, Van Broeckhoven C, Vandenberghe A: Mutations in the myelin protein zero gene associated with Charcot-Marie-Tooth disease type 1B. Hum Mutat 1995;6:50–54.
66 Silander K, Meretoja P, Nelis E, Timmerman V, Van Broeckhoven C, Savontaus ML: A de novo duplication in 17p11.2 and a novel mutation in the P0 gene in two Déjérine-Sottas syndrome patients. Hum Mutat, in press.
67 Blanquet-Grossard F, Pham-Dinh D, Dautigny A, Latour P, Bonnebouche C, Diraison P, Chapon F, Chazot G, Vandenberghe A: Charcot-Marie-Tooth type 1B neuropathy: A mutation at the single glycosylation site in the major peripheral myelin glycoprotein P0. Hum Mutat, in press.
68 Rautenstrauss B, Nelis E, Grehl H, Pfeiffer RA, Van Broeckhoven C: Identification of a de novo insertional mutation in P0 in a patient with a Déjérine-Sottas syndrome (DSS) phenotype. Hum Mol Genet 1994;3:1701–1702.
69 Bellone E, James R, Mandich P, Nelis E, Lamb LD, Van Broeckhoven C, Ajmar F: Identification of a 4 bp deletion (1560del4) in P0 gene in a family with severe Charcot-Marie-Tooth disease. Hum Mutat, in press.
70 Nelis E, Simokovic S, Timmerman V, Löfgren A, Backhovens H, De Jonghe P, Martin J, Van Broeckhoven C: Mutation analysis of the connexin32 (Cx32) gene in Charcot-Marie-Tooth neuropathy type 1: Identification of five new mutations. Hum Mutat, in press.