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Identification and *in silico* analysis of 14 novel *GJB1*, *MPZ* and *PMP22* gene mutations

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Duplication within the chromosome 17p11.2 (CMT1Adup), peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ) and gap junction β1-protein (GJB1) gene mutations are frequent causes of the Charcot-Marie-Tooth disease (CMT). A large number of mutations in these genes are listed in databases. Sequence variants identified in patients are frequently reported as mutations without further evaluation. We analyzed 250 consecutively recruited unrelated Austrian CMT patients for CMT1Adup by microsatellite marker typing, real-time PCR or MLPA, and found 79 duplications (31.6%). The coding regions of the PMP22, MPZ and GJB1 genes were analyzed by direct sequencing in the remaining patients; 28 patients showed mutations, 14 of which were novel. We scored the pathogenicity of novel missense mutations by segregation studies and by their exclusion in control samples. Our comprehensive literature study found that up to 60% of the reported mutations in these genes had not been evaluated regarding their pathogenicity, and the PANTHER bioinformatics tool was used to score novel and published missense variants. The PANTHER program scored known polymorphisms as such, but scored ∼82–88% only of the published and novel mutations as most likely deleterious. Mutations associated with axonal CMT were less likely to be classified as deleterious, and the PMP22 S72L mutation repeatedly associated with severe CMT was classified as a polymorphism using default parameters. Our data suggest that this *in silico* analysis tool could be useful for assessing the functional impact of DNA variations only as a complementary approach. The CMT1Adup, GJB1, MPZ and PMP22 mutation frequencies were in the range of those described in other CMT patient collectives with different ethnical backgrounds.
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Introduction

The Charcot-Marie-Tooth disease (CMT; MIM 118220), also known as hereditary motor and sensory neuropathy, occurs with an estimated frequency of 1:2500 individuals.[1] The main clinical characteristics are slowly progressive distal muscle weakness and wasting first of the lower and later

usually also of the upper limbs, sensory loss and foot deformity. CMT has been subdivided according to the nerve electrophysiological criteria into two main groups:[2–4] CMT1, which is characterized by reduced motor nerve conduction velocities (MNCV) of the median nerve (MNCV <38 m/s) and nerve demyelination, and the axonal variant, CMT2, with MNCV being almost normal (MNCV >38 m/s), but with reduced amplitudes of compound motor-evoked potentials. An additional classification of intermediate CMT has been suggested in cases with MNCV between 25 and 45 m/s.[5] CMT1 is frequently associated with duplications on the chromosome 17p11.2–p12 (CMT1A), which contains the peripheral myelin protein 22 gene (PMP22; MIM 601097).[6] Further mutations in 36 genes have been described in CMT patients (http://www.molgen.ua.ac.be/CMTMutations/default.cfm). Mutations in the gap junction β-1 protein located on chromosome Xq13.1 (GJB1 or Connexin 32, Cx32; MIM 304040) may lead to CMT type X1 (CMTX1), and electrophysiology may suggest both demyelinating and/or axonal nerve damage, thus resulting in either a CMT1, CMT2 or in an intermediate phenotype.[7] Myelin protein zero (MPZ or P0; MIM 159440) mutations are associated with CMT1, but can also cause CMT2 or the Dejerine–Sottas syndrome (DSS).[8,9] The PMP22 mutations can cause CMT1, DSS, congenital hypomyelination (CHN) and, if inactivated, can also cause hereditary neuropathy with a liability to pressure palsies (HNPP).[10]

We examined 250 unrelated Austrian CMT patients for the CMT1A duplication 17p11.2–p12. A total of 171 individuals without duplication were screened for mutations in the *GJB1*, *MPZ* and *PMP22* genes. We report on the mutation frequency distribution in this population, and describe 14 novel sequence variations detected in patients. The pathogenicity of novel and published mutations was also evaluated using the recent bioinformatics program, PANTHER.[11]

Patients, materials and methods
Patients
Patients diagnosed with CMT were referred from neurological departments throughout Austria, and were consecutively included in this study. Written informed consent was obtained from all the tested persons according to the Declaration of Helsinki. Most of the patients were of Austrian ethnicity. The clinical criteria of CMT required at least the presence of distinct gait abnormalities, because of weakness of the foot extensors. On the basis of the MNCV measurements, 85 patients were classified as CMT1, and 29 patients as CMT2. In the remaining cases, the clinical data were incomplete for further subclassification.

Mutation analysis
DNA was extracted from peripheral blood samples using an automated extractor according to the manufacturer's

protocols (GenoM 48, Qiagen, Vienna, Austria). Samples were analyzed for the presence of a 1.4-Mb CMT1A duplication on chromosome 17p by genotyping either five polymorphic microsatellite markers (D17S2220, D17S2224, D17S2226, D17S2227 and D17S2230),[12] real-time PCR or multiplex ligation-dependent probe amplification (SALSA MLPA kit P033(B)). The coding regions and splice sites of the *GJB1*, *MPZ* and *PMP22* genes were amplified by PCR in the index patients (Supplementary Table 1). After the detection of a mutation in a proband, a number of clinically affected or healthy relatives were tested for this mutation, when available. The sequencing reactions were analyzed on the ABI 3100 DNA sequencer, using the BigDye terminator mix (Applied Biosystems, Vienna, Austria). PCR amplicons from anonymous healthy controls were analyzed for the presence of novel mutations, using denaturing high performance liquid chromatography.

Bioinformatics analysis
We used the *in silico* sequence alignment program, ClustalW, and the evolutionary model program, PANTHER,[11] to estimate the functional consequences of published and novel missense mutations in these genes (http://www.pantherdb.org/). The mutation nomenclature is based on protein reference sequences, such as MIM NP_000157 (GJB1) and MIM NP_000295 (PMP22). Consistent with the CMT mutation database (http://www.molgen.ua.ac.be/CMTMutations/default.cfm), the nomenclature describing MPZ mutations was based on the MPZ sequence D10537 (http://www.ncbi.nlm.nih.gov/entrez/), as published by Hayasaka *et al*,[13] and not on the MPZ RefSeq NM_000530 (http://www.ncbi.nlm.nih.gov/entrez/).

Results and discussion
Among the 250 unrelated CMT patients, 79 of them (31.6%) harbored the CMT1A duplication. Of these, 43 patients were classified as CMT1, and the MNCV data were lacking for 36 patients. One patient was identified to harbor the typical HNPP deletion.

A subsequent mutation analysis of GJB1, MPZ and PMP22 in CMT patients without CMT1Adup revealed 28 heterozygous or hemizygous mutations. We identified 4 novel truncating or frameshifting, and 10 novel missense mutations, which we considered pathogenic (Table 1). Neither of these mutations was detected among the 93 controls (blood donors).

Disease onset was reported in the first to third decade of life in all patients with novel mutations, except for a female with the MPZ mutation, p.D224Y. Four of six patients with the MPZ mutations were classified as CMT1. The MPZ mutations, p.I30S and p.R185AfsX66, were associated with a severe, early-onset demyelinating phenotype similar to those observed earlier in patients with mutations of codon 30, and the abnormal C-terminal

Table 1 Clinical and genetic findings of CMT patients with novel mutations in the *GJB1*, *MPZ* and *PMP22* genes

ID	Sex	Family history	Age of onset (years)	Age at examination (years)	mNCV/ CMAP (m/s/mV)	sNCV/ SNAP (m/s/µV)	UL weakness	LL weakness	Tendon reflexes	Sensory impairment	Pes cavus	Additional findings	Gene	Nucleotide position	aa change
1	M	S[a]	5–6	41	48/1.9	37/7.2	+	+++	N	ND	+++	No	*GJB1*	c.11C>A	p.T4K
2	M	F (4)	7–8	28	ND	ND	+++	+++	A	ND	+++	Cerebellar signs in one affected individual	*GJB1*	c.94A>G	p.R32G
3	F	F (3)	6	31	40/3.6	NR	+	+	D	ND	+	No	*GJB1*	c.155delT	p.I52TfsX31
4	F	F (2)	5–6	50	31/0.1	NR	++	+++	A	ND	++	No	*GJB1*	c.278T>A	p.M93K
5	M	F (8)	ND	68	ND	ND	+++	+++		ND	+++	No	*GJB1*	c.437A>C	p.E146A
6	M	F (2)	Newborn	18	34/14	38/5	–	+++	D/A	Yes	+++	No	*GJB1*	c.592T>G	p.S198A
7	F	F (4)	16	46	35/1.6	37/1	+	+++	A	Yes	ND	No	*GJB1*	c.829_904 del14	p.S277GfsX128
8	F	F	2	9	4/0.8	NR	++	++	A	Yes	+	No	*MPZ*	c.89T>G	p.I30S
9	F	S[a]	10	37	53/ND	58/ND	ND	ND	ND	ND	ND	No	*MPZ*	c.98A>T	p.Y33F
10	F	F	5–6	38	16/6	27/9	+	+	A/D	Yes	++	No	*MPZ*	c.148T>G	p.C50G
11	M	F (5)	Childhood	27	29/2	33/ND	–	++	D	Yes	+++	No	*MPZ*	c.298C>T	p.Q100X
12	M	S de novo	1	1.5	11/ND	NR	++	++	D	No	no	Macro-cephalia	*MPZ*	c.553delC	p.R185AfsX66
13	M	F (3)	43	45	34/12	36/17	–	–	D	Yes	+++	No	*MPZ*	c.670G>T	p.D224Y
14	F	F	10	52	39/7.0	38/10	–	+	N/A	Yes	++	Gait ataxia	*PMP22*	c.332T>C	p.M111T

Sex: F, female; M, male; Family history: S, sporadic; F, familial; if F is the number of affected relatives with the same mutation.
aParents not available for examination; mNCV, motor nerve conduction velocities of median nerve; sNCV, sensory nerve conduction velocities of median nerve; CMAP, compound motor action potential of abductor pollicis brevis muscle; SNAP, sensory nerve action potential of median nerve; ND, no data; NR, no response; UL, upper limbs; LL, lower limbs; +, mild; ++, moderate; +++, severe; −, absent; N, normal; A, absent; D, diminished; aa, amino acid.

p.R185AfsX66 protein[14,15] further confirming the observed genotype–phenotype correlation of the MPZ mutations.[8]

One CMTX patient additionally showed cerebellar ataxia, the significance of which is unclear at present. Ataxia did not segregate within the family, and the most common genetic causes of ataxia (Friedreich ataxia, SCA 1, 2, 3 and 6) were excluded. The individual carrying the novel PMP22 mutation had median MNCV of 39 m/s and gait ataxia.

The identified mutations: p.R22Q, p.R75W, p.W77S, p.R107W, p.R142Q, p.V181M and p.N205S in GJB1; p.S20F, p.D60H, p.I62M and p.S78L in MPZ; and pS72L, c.281-282insG and p.T118M in PMP22 were described earlier and are listed in the European CMT Consortium Mutations Update database, accessed at http://www.molgen.ua.ac.be/CMTMutations/default.cfm.

Truncating and frameshifting mutations

The GJB1 p.I52TfsX31 mutation causes an early truncation of the 283-amino acid wild-type protein. The p.Q100X in exon 3 of MPZ most likely causes a nonsense-mediated decay, and segregates with the disease in a large pedigree. The c.553delC deletion in exon 4 of MPZ results in a frameshift with a large abnormal C-terminal peptide sequence (p.R185AfsX66). This mutation was found to occur *de novo* in our patient. An MPZ mutation, c.554delG, with the same consequence at the protein level has been described as a cause of CMT earlier.[15] The deletion of 14 nucleotides, c.829_843, in GJB1 removes the last 6 amino acids and adds 128 abnormal amino acids C terminally (p.S277GfsX128), and is most likely to interfere with the normal connexin structure.

Missense mutations

The high degree of conservation of the identified mutated residues in GJB1, MPZ and PMP22 is represented in Figure 1, and these missense changes are non-conservative (Table 1). Distinct mutations within three mutated GJB1 codons, p.S198P, p.M93V and p.E146K, have been described earlier in CMT patients.[16] Notably, the MPZ mutation, p.C50G, is predicted to cause loss of a disulfide bridge in MPZ.[17] The MPZ mutation, p.I30S, affects the highly conserved, first amino acid residue after the cleavage of a 29 amino acid signal peptide. Deletion of isoleucin-30, as well as changes p.I30T and p.I30M have been described in patients with CMT1 and DSS. However, family studies regarding the MPZ mutation, p.Y33F, and the GJB1 mutation, p.T4K, were not feasible.

The generally used criteria for scoring the pathogenicity of identified missense variants are the absence of identified sequence variants among healthy controls and the segregation of mutations with the disease within families. In addition, co-investigated patient cohort samples are usually considered to serve as controls for determining the frequency of mutations. However, especially in simplex

a

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                                               4                              32
Homo sapiens/NP_000157                MNWTGLYTLLSGVNRHSTAIGRVWLSVIFIFRIMVLVVAA
Rattus norvegicus/NP_058947           MNWTGLYTLLSGVNRHSTAIGRVWLSVIFIFRIMVLVVAA
Mus musculus/NP_032150                MNWTGLYTLLSGVNRHSTAIGRVWLSVIFIFRIMVLVVAA
Equus caballus/NP_001075360           MNWTGLYTLLSGVNRHSTAIGRVWLSVIFIFRIMVLVVAA
Bos taurus/NP_776494                  MNWTGLYTLLSGVNRHSTAIGRVWLSVIFIFRIMVLVVAA
Xenopus tropicalis/NP_001001240       MNWAGLYAILSGVNRHSTSIGRIWLSVVFIFRIMVLVVAA

                                                                    93
Homo sapiens/ NP_000157               NSVCYDQFFPISHVRLWSLQLILVSTPALLVAMHVAHQQH
Rattus norvegicus/ NP_058947          NSVCYDHFFPISHVRLWSLQLILVSTPALLVAMHVAHQQH
Mus musculus/ NP_032150               NSVCYDHFFPISHVRLWSLQLILVSTPALLVAMHVAHQQH
Equus caballus/ NP_001075360          NSVCYDHFFPISHVRLWSLQLILVSTPALLVAMHVAHQQH
Bos taurus/ NP_776494                 NSVCYDHFFPISHVRLWSLQLILVSTPALLVAMHVAHQQH
Xenopus tropicalis/ NP_001001240      NSVCYDHFFPISHIRLWALQLIIVSTPALLVAMHVAHLQH

                                                                146
Homo sapiens/ NP_000157               HGDPLHLEEVKRHKVHISGTLWWTYVISVVFRLLFEAVFM
Rattus norvegicus/ NP_058947          HGDPLHLEEVKRHKVHISGTLWWTYVISVVFRLLFEAVFM
Mus musculus/ NP_032150               HGDPLHLEEVKRHKVHISGTLWWTYVISVVFRLLFEAVFM
Equus caballus/ NP_001075360          HGDPIHLEEVKRHKVHISGTLWWTYVISVVFRLLFEAAFM
Bos taurus/ NP_776494                 HGDPLHLEEVKRHKVHISGTLWWTYVISVVFRLLFEAAFM
Xenopus tropicalis/ NP_001001240      HVKDQELAEVKKHKVKISGTLWWTYIISVFFRIIFEAAFM

                                                                    198
Homo sapiens/ NP_000157               AMVRLVKCDVYPCPNTVDCFVSRPTEKTVFTVFMLAASGI
Rattus norvegicus/ NP_058947          AMVRLVKCEAFPCPNTVDCFVSRPTEKTVFTVFMLAASGI
Mus musculus/ NP_032150               AMVRLVKCEAFPCPNTVDCFVSRPTEKTVFTVFMLAASGI
Equus caballus/ NP_001075360          AMVRLVKCDAYPCPNTVDCFVSRPTEKTVFTVFMLAASGI
Bos taurus/ NP_776494                 AMVRLVKCDAYPCPNTVDCFVSRPTEKTIFTVFMLAASGI
Xenopus tropicalis/ NP_001001240      SMIRLVKCDAYPCPNTVDCFVSRPTEKTIFTVFMLVASGF
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b

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                                             30 33                  50
Homo sapiens/ NP_000521               AVLLFSSLVLSPAQAIVVYTDREVHGAVGSRVTLHC
Rattus norvegicus/ NP_058723          AALLFSSLVLSPTLAIVVYTDREVYGAVRSQVTLHC
Mus musculus/ NP_032649               AALLFSSLVLSPALAIVVYTDREIYGAVGSQVTLHC
Equus caballus/ NP_001075363          AALLFSSLVLSPAQAIVVYTDKEVYGAVGSRVTLHC
Canis familiaris/ENSCAFP00000019099   AVLLFSSLVLPPAQAIVVYTDREVHGAVGSRVTLHC
Bos taurus MYP0/ ENSBTAP00000045141   AALLFSSLVLSPVQAIVVYTDKEVHGAVGSQVTLYC

                                                            224
Homo sapiens/ NP_000521               SAMEKGKLHKPGKDASKRGRQTPVLYAMLDHSRSTK
Rattus norvegicus/ NP_058723          SAMEKGKFHKSSKDSSKRGRQTPVLYAMLDHSRSTK
Mus musculus/ NP_032649               SAMEKGRFHKSSKDSSKRGRQTPVLYAMLDHSRSTK
Equus caballus/ NP_001075363          SAMEKGKLHKPGKDTSKRGRQTPVLYAMLDHSRSTK
Canis familiaris/ENSCAFP00000019099   SAMEKGKLHKAGKDTSKRGRQTPVLYAMLDHSRSTK
Bos taurus MYP0/ ENSBTAP00000045141   SAMEKGKLHKTAKDASKRGRQTPVLYAMLDHSRSTK
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c

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                                                 111
Homo sapiens/ NP_000295               IFQILAGLCVMSAAAIYTVRHPEWHLNSD
Rattus norvegicus/ NP_058733          VFQILAGLCVMSAAAIYTVRHSEWHVNND
Mus musculus/ NP_032911               FFQILAGLCVMSAAAIYTVRHSEWHVNTD
Equus caballus/ NP_001075405          IFQILAGLCVMSAASIYTVRHPEWHLDSA
Danio rerio/ NP_958468                VFQILASLFVMSGAIIYTVMSPQWKNDSD
Canis familiaris/ENSCAFP00000026442   VFQILAGLCVMSAASIYTVRHPEWHFNSD
Gallus gallus/ ENSGALP00000002076     IFQILAGLCVMSGAAIFTVRHTDWHEASE
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Figure 1 Conservation of mutated amino acids according to a ClustalW sequence alignment. (**a**) GJB1 (MIM NP_000157), (**b**) MPZ (MIM NP_000521) and (**c**) PMP22 (MIM NP_000295). Mutated residues are boxed and labeled by codon number.

cases or small families, and when investigating small patient cohorts, missense variants may not be interpreted unambiguously. Functional studies of identified missense changes are often time consuming, produce ambiguous results or are not feasible. Therefore, we choose to analyze our data further by using the PANTHER computer program

as a complementary approach to differentiate between putative mutations and polymorphisms.

The PANTHER program is a recent development that is used to score sequence variants *in silico*, and it makes use of the increasing number of database entries regarding homologous and orthologous proteins of human disease

proteins for evaluating the degree of conservation of amino acid residues, as well as for indicating the potential deleterious effects of missense changes (sub-PSEC scores).[18] The PANTHER sub-PSEC scores are continuous values from 0 (neutral) to approximately −10 (most likely to be deleterious).

The sub-PSEC score for novel mutations performed using the PANTHER software was in the significant range (<-3 (eg, −7)) for 3 of the 5 GJB1, 3 of the 4 MPZ and for the novel PMP22 mutation, further indicating that at least 7–10 changes have a functional consequence on the protein level (Table 2).

We further evaluated our findings by a PANTHER analysis of published missense variations in these genes (http://www.molgen.ua.ac.be/CMTMutations/default.cfm). All the missense changes described earlier as polymorphisms were scored as such by the PANTHER program (Table 2). However, 28 (68 of 214), 12 (10 of 83) and 18% (6 of 33) of the published disease-causing mutations and mutations detected in GJB1, MPZ and PMP22, respectively, in this study, showed a sub-PSEC score >-3 (eg, −2), that is, these mutations were not classified as disease causing. However, there was a trend apparent that the $P_{deleterious}$ values were correlated with phenotypic severity, generally suggesting that PANTHER can discriminate between neutral and functional mutants (Table 2). Mutations associated with CMT2, which were thought to result in a loss of function, were less likely to be classified as deleterious.

Further limits of the PANTHER computer program also became apparent as exemplified by the analysis of the PMP22 mutation, S72L; this mutation was identified in at least 12 independent studies as one of the most severe CMT mutations (leading to CHN and wheelchair dependency in early childhood and frequently to premature death) among all known CMT-associated genes which contrasts with the PANTHER sub-PSEC score of −2.93 of a disease-unrelated variation. Furthermore, a PANTHER sub-PSEC score of −3.98 was obtained for the T118M PMP22 mutation, which was repeatedly reported in healthy individuals. Other sequence variations might also be misclassified using this line of analysis.

We further investigated whether missense changes, which were not scored as mutations by the PANTHER, were also less supported as such by conventional criteria. We searched all references regarding the MPZ, PMP22 and GJB1 mutations as given in the European CMT Consortium Mutations database for specific information regarding co-segregation within families (number of blood-related individuals), and the number of unrelated controls excluded to harbor the mutation (Table 2). Notably, we found that references contained no information regarding co-segregation within families (number of individuals), and the number of controls excluded to harbor the mutation in up to 60% of cases (Table 2).

For 5/10 (50%) MPZ, 4/6 (66.7%) PMP22 and 8/13 (61.5%) GJB1 variations listed as mutations, but scored as most likely non-deleterious by PANTHER, co-segregation studies were not available or had not been performed, in contrast to 32/74 (43%) MPZ and 11/27 (40.7%) PMP22 variants listed as mutations and scored as most likely

Table 2 Newly designed primer sequences for amplification and sequencing of GJB1, MPZ and PMP22

GJB1			
Exon 2 PCR-Primer	Cx32part1f	TGAGGCAGGATGAACTGGACAGGT	990 bp
	Cx32part3r	TAGCCAGGGAAGGAAGGTTT	
Internal sequencing primer	Cx32part2f	ATCTCCCATGTGCGGCTGTGGTCC	
	Cx32part2r	GATGATGAGGTACACCACCT	
MPZ			
Exon 1	MPZ Ex1f	CCCGTTCAGTTCCTGGT	189 bp
	MPZ Ex1r	GCTGAGAGACACCTGAGTCC	
Exons 2–6	MPZ Ex2f	CTTCCTCTGTATCCCTTACTG	1694 bp
	MPZ Ex5/6r	TAGCTCCATCTCGATGACCA	
Internal sequencing primer	MPZ Ex3r	GCCTGAATAAAGGTCCTTAGGC	
	MPZ Ex4f	GGAGTCCTACATCCTCAATGCAG	
	MPZ Ex4r	CCCACCCACTGGAGTAGTCTCCG	
	MPZ Ex5/6f	CAAGGGGAAGAGGAAGCTGT	
PMP22			
Exon 1	F	CTCCTCGCAGGCAGAAACTC	202 bp
	R	CTGAACCAGCAGGAGCACGGG	
Exon 2	F	TCAGGATATCTATCTGATTCTC	156 bp
	R	AAGCTCATGGAGCACAAAACC	
Exon 3	F	TGGCCAGCTCTCCTAAC	217 bp
	R	CACCCCGCTTCCACATG	
Exon 4	F	GCCATGGACTCTCCGTC	249 bp
	R	CCTATGTACGCTCAGAG	

deleterious by PANTHER. There were 126/207 (60.9%) GJB1 mutations with a deleterious PANTHER score without co-segregation studies.

The mechanism by which the *MPZ* and *PMP22* mutations affects protein function remain to be determined in most instances, but may involve a gain of function by aberrant interactions within the MPZ tetramer and within the PMP22 dimer/multimer itself or between MPZ and PMP22, as well as a loss of function by expression of an unstable protein that is rapidly degraded or by an interference with protein translation. These different mechanisms are probably not detected with the same sensitivity, using the approach of evolutionary conservation of residues. However, our analysis suggests that obtaining a sub-PSEC value below the −3 cutoff for sequence variants, at least in the analyzed genes, supports the assumption of a mutation's pathogenicity. We also report that a large number of published MPZ, PMP22 and GJB1 variants have not been evaluated for their pathogenicity. Taken together, our data suggest that at least some of the missense variants contained in the database might represent rare polymorphisms including the MPZ mutation, p.Y33F, and the GJB1 mutation, p.T4K, that are identified in this study.

The frequency of the 17p11.2–p12 duplication in our CMT patient cohort (31.6%) is in the range of other studies in different ethnical populations.[6,7,15,21–26] The mutation frequencies of 7.6, 4 and 2% for GJB1, MPZ and PMP22 mutations, respectively, in our CMT patient cohort were comparable with those reported in a number of other studies. Screening of further known CMT genes is ongoing to identify the genetic causes in the remaining 145 CMT patients.

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