Demyelinating and axonal features of Charcot–Marie–Tooth disease with mutations of myelin-related proteins (PMP22, MPZ and Cx32): a clinicopathological study of 205 Japanese patients

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Summary
Three genes commonly causing Charcot–Marie–Tooth disease (CMT) encode myelin-related proteins: peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ) and connexin 32 (Cx32). Demyelinating versus axonal phenotypes are major issues in CMT associated with mutations of these genes. We electrophysiologically, pathologically and genetically evaluated demyelinating and axonal features of 205 Japanese patients with PMP22 duplication, MPZ mutations or Cx32 mutations. PMP22 duplication caused mainly demyelinating phenotypes with slowed motor nerve conduction velocity (MCV) and demyelinating histopathology, while axonal features were variably present. Two distinctive phenotypic subgroups were present in patients with MPZ mutations: one showed preserved MCV and exclusively axonal pathological features, while the other was exclusively demyelinating. These axonal and demyelinating phenotypes were well concordant among siblings in individual families, and MPZ mutations did not overlap among these two subgroups, suggesting that the nature and position of the MPZ mutations mainly determine the axonal and demyelinating phenotypes. Patients with Cx32 mutations showed intermediate slowing of MCV, predominantly axonal features and relatively mild demyelinating pathology. These axonal and demyelinating features were present concomitantly in individual patients to a variable extent. The relative severity of axonal and demyelinating features was not associated with

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particular Cx32 mutations. Median nerve MCV and overall histopathological phenotype changed little with disease advancement. Axonal features of diminished amplitudes of compound muscle action potentials (CMAPs), axonal loss, axonal sprouting and neuropathic muscle wasting all changed as disease advanced, especially in PMP22 duplication and Cx32 mutations. Median nerve MCVs were well maintained independently of age, disease duration and the severity of clinical and pathological abnormalities, confirming that median nerve MCV is an excellent marker for the genetically determined neuropathic phenotypes. Amplitude of CMAPs was correlated significantly with distal muscle strength in PMP22 duplication, MPZ mutations and Cx32 mutations, while MCV slowing was not, indicating that clinical weakness results from reduced numbers of functional large axons, not from demyelination. Thus, the three major myelin-related protein mutations induced varied degrees of axonal and demyelinating phenotypic features according to the specific gene mutation as well as the stage of disease advancement, while clinically evident muscle wasting was attributable to loss of functioning large axons.

**Keywords:** Charcot–Marie–Tooth disease; PMP22; MPZ; Cx32

**Abbreviations:** CK = creatine kinase; CMAP = compound muscle action potential; CMT = Charcot–Marie–Tooth disease; Cx32 = connexin 32; DL = distal latency; MCV = motor nerve conduction velocity; MPZ = myelin protein zero; PMP22 = peripheral myelin protein 22; SCV = sensory nerve conduction velocity; SNAP = sensory nerve action potential

**Introduction**

Charcot–Marie–Tooth disease (CMT) refers to a pathologically and genetically heterogeneous group of motor and sensory neuropathies characterized by slowly progressive weakness, muscle atrophy and sensory impairment, all most marked in the distal part of the legs. Two major phenotypes have been distinguished (Dyck and Lambert, 1968; Buchthal and Behse, 1977; Harding and Thomas, 1980). Type 1 (CMT1), the demyelinating form of CMT, results in a marked decrease in nerve conduction velocity (NCV) and segmental demyelination of peripheral nerves; type 2 (CMT2), the axonal form of CMT, shows primarily axonal involvement, with normal or slightly decreased NCV, as well as nerve fibre loss and axonal sprouting. In addition, a group showing features intermediate between those of types 1 and 2 has also been described (Bradley et al., 1977). Many causative genes have been identified, prompting proposals for a new classification (Harding, 1995; Kamholz et al., 2000; Reilly, 2000). Heterozygous duplication of the locus for the peripheral nerve myelin protein 22 (PMP22) gene on chromosome 17p11.2 is found in most patients with demyelinating CMT1A (Lupski et al., 1991; Raeymaekers et al., 1991; Matsunami et al., 1992; Patel et al., 1992; Timmerman et al., 1992). Occasionally, a point mutation in the PMP22 gene rather than duplication is found in CMT1A patients (Nelis et al., 1996; Kovach et al., 1999). CMT1B, another demyelinating form, has been found to result from mutations in the myelin protein zero (MPZ) gene (Hayasaka et al., 1993). Disease in another group of patients results from mutations of the connexin 32 (Cx32) gene (Bergoffen et al., 1993; Ionasescu et al., 1994, 1996). These three genes encoding myelin-related proteins are the major causes of CMT with demyelination. Recently, rare gene mutations in the early growth response protein (ERG2), myotubularin-related protein 2 (MTMR2), N-myc downstream-regulated gene 1 (NDRG-1), periaxin (PRX) and ganglioside-induced differentiation-associated protein 1 (GDAP1) genes have been found in patients with the demyelinating form (Warner et al., 1998; Bolino et al., 2000; Kalaydjieva et al., 2000; Guibot et al., 2001; Nagarajan et al., 2001; Yoshihara et al., 2001; Baxter et al., 2002). Several genes causing CMT type 2 have also been identified, including neurofilament L (NF-L) the kinesin motor superfamily (KIF1Bb) and lamin A/C nuclear envelope proteins, as well as GDAP1 (Mersiyanova et al., 2000; Zhao et al., 2001; De Sandre-Giovannoli et al., 2002; Cuesta et al., 2002).

CMT phenotypes caused by different gene abnormalities have been widely analysed, but no firm consensus has been established (Boerkoel et al., 2002). In patients with Cx32 mutations, some authors considered the demyelinating process to predominate (Scherer, 1999; Scherer and Fischbeck, 1999; Tabaraud et al., 1999; Gutierrez et al., 2000), while others favoured a primarily axonal neuropathy (Hahn et al., 1990, 2001; Birouk et al., 1998; Senderek et al., 1999). As for MPZ mutations, several families with an axonal phenotype have been reported (Gabreels-Festen et al., 1996; Marrous et al., 1998; Chapon et al., 1999; Boerkoel et al., 1999, 2001; Boerkoel et al., 2002), suggesting a subgroup of patients with MPZ mutations causing a primarily axonal neuropathy. Wide variation has been shown in pathological findings, nerve conduction data, axonal and demyelinating severity, associated symptoms and prognosis in patients with MPZ, Cx32 and PMP22 gene abnormalities (Killian et al., 1996; Birouk et al., 1997, 1998; Thomas et al., 1997; Senderek et al., 1999; Dubourg et al., 2001a; Boerkoel et al., 2002). Phenotypic variation and the underlying gene abnormalities remain to be defined.

Axonal involvement has been reported in CMT1A, a demyelinating form (Dyck et al., 1989; Garcia et al., 1998; Krajewski et al., 2000), as evidenced by decreased amplitude of muscle action potentials and by pathological abnormalities
In CMT1 patients, axonal involvement progressed in a time-dependent manner during a longitudinal study (Dyck et al., 1989). Furthermore, conduction velocity and amplitude or neuropathic deficits at the first and last examinations in CMT1 were significantly correlated, suggesting an association between early slowing of conduction and subsequent neuropathic disability (Dyck et al., 1989). These observations suggest that axonal involvement related to disease progression in demyelinating neuropathy is clinically important. However, demyelinating and axonal features that alter as disease progresses have not been assessed conclusively in CMT, particularly in forms other than CMT1A.

In addition, most studies of CMT phenotypic–genotypic variation have been performed in Caucasian populations; thus, features of CMT phenotypic–genotypic variation in Asian populations have not been elucidated. The frequency distribution of the breakpoint for PMP22 duplication at the CMT1A-REP (repeat) in 17p11.2 has been documented to be very similar among Caucasian and Asian populations (Yamamoto et al., 1997), while the prevalence of CMT1A in the Japanese population has been considered to be extremely low compared with frequencies in Caucasians, although definitive comparative epidemiological studies have not been reported.

We therefore conducted a clinical, electrophysiological, histopathological and genetic study of 205 Japanese CMT patients with one of the three most common causative gene abnormalities, viz. abnormalities of PMP22, MPZ and Cx32, which involve major myelin-related proteins. We focused particularly on the demyelinating and axonal phenotypic features of these CMT patients.

**Patients and methods**

**Patients and DNA diagnosis**

Two hundred and five CMT patients from 124 families with a DNA diagnosis of PMP22 duplication (118 patients from 74 families), MPZ mutations (45 patients from 26 families) or Cx32 mutations (42 patients from 24 families; male only) were registered by the Study Group for Hereditary Neuropathy in Japan, working under the auspices of the Ministry of Health, Labour and Welfare of Japan. In the case of Cx32 mutations, gender effects on phenotypic expression are known to be profound (Nicholson and Nash, 1993; Birouk et al., 1998; Dubourg et al., 2001b), so only male patients were selected for our genotype-phenotype analysis. DNA analysis was performed in the Department of Neurology, Nagoya University Graduate School of Medicine, the Department of Pediatrics, Yamagata University School of Medicine, the Department of Neurology, Kagoshima University School of Medicine, or the Department of Neurology, Osaka University School of Medicine. In most cases, the PMP22 duplication was detected by Southern analysis, probing with PMP22 cDNA, the polymorphic markers VAW409R3 and EW401, and the CMT1A-REP fragments (Ikegami et al., 1997; Yamamoto et al., 1997, 1998). Hybridization with the probes pNEA102, pHK1.0P and pHK5.2P, mapping within the CMT1A-REP, was used to determine the location of crossover breakpoints, which were clustered in a 700-base pair (bp) region of the CMT1A-REP. Quantitation of hybridized signals for each band was performed to evaluate duplicate gene dosage using a phosphor-image analyser (BAS-2000II; Fujix, Tokyo, Japan). In some cases, fluorescence in situ hybridization (FISH) was employed to detect PMP22 gene duplication.

A non-isotopic RNase cleavage assay (NIRCA) was employed as a screening test for MPZ and Cx32 mutations using a method described previously (Yoshihara et al., 2000). The MPZ and Cx32 genes, separated into three and two fragments respectively, were amplified with the polymerase chain reaction (PCR). Sequences of the forward (F) and reverse (R) primers were as follows:

For the MPZ gene: F1, 5'-CTA GGG ATT TTA AGC AGG TTC C-3' and R1, 5'-ATT GCT GAG AGA CAC CTG AGT CC-3' for exon 1; F2, 5'-CCA TAG GTG CAT CTG ATT CC-3' and R2, 5'-CCT CCT TAG CCC AAT TTA TC-3' for exon 2; and F3, 5'-CAG CTG TGT TCT CAT TAG GGT CCT C-3' and R3, 5'-GCT CAT CCT TTC GTA GCT CCA TCT C-3' for exons 3–6. For the Cx32 gene: F4, 5'-AGT GAC GAG GAG GTG TGA TAT GTC-3' and R4, 5'-AGG GGT AGA CGT CGC ACT TGA C-3' for part 1 of exon 2; and F5, 5'-TTT GAG GCC GTC TTC ATG TAT GTC-3' and R5, 5'-AGT AGC CAG GGA AGG GTT TTG-3' for part 2 of exon 2.

The bacteriophage T7 promoter sequence 5'-TAA TAC GAC TCA CTA TAG GG-3' was attached to each primer at the 5' end. PCR amplification was performed using AmpliTaq Gold (Perkin Elmer, Wellesley, MA, USA). Initial amplification conditions were 95°C for 9 min for denaturation, followed by 35 cycles of denaturing at 95°C for 1 min, annealing at 60°C for 1 min and elongation at 72°C for 2 min. NIRCA was performed using a MutationScreener kit (Ambion, Austin, TX, USA) according to the manufacturer’s protocol. This assay system is based on the properties of the RNase enzyme that cleaves single base-pair mismatches of hybridized RNA duplexes produced in vitro by generating sense and antisense transcripts of each PCR product using the specific primer pairs. The bacteriophage RNA T7 promoter sequence integrated into the forward and reverse primers allowed the PCR products to be converted to RNA. Sense and antisense RNA transcripts were hybridized, and RNA–RNA hybrids were then treated with an optimized RNase mixture (RNase I and RNase T1) to cleave the duplexes at the mismatch positions. RNase-digested products were electrophoresed through 2.5% agarose gels. PCR products were purified from the unreacted primers and nucleotides using a QIAquick PCR Purification kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s protocol. Duplicate amplifications analysed by NIRCA were performed to avoid false-positive results arising from PCR errors. Purified products...
were sequenced directly using a Thermo Sequenase Cy5.5 terminator cycle sequencing kit (Amersham Biosciences, Piscataway, NJ, USA) and analysed with a GeneRapid sequencer system (Amersham, Pharmacia). In some cases, FISH was employed to detect PMP22 gene duplication. As the patients with point mutations of the PMP22 gene are not included in this paper, ‘PMP22 mutation’ means ‘PMP22 duplication’.

Informed consent was granted by subjects beforehand according to the guidelines of the Ethics Committee of Nagoya University Graduate School of Medicine or those of the regional ethics committee for each institution.

**Clinical assessment**

Clinical information was assessed in a standardized manner, including motor and sensory impairment, deep tendon reflexes, muscular atrophy or hypertrophy, foot deformity and autonomic impairment. Weakness was assessed in proximal muscles (deltoid, biceps, triceps muscles in the arm; iliopsoas, quadriceps muscles in the legs) as well as distal muscles (thenar, interosseous, finger flexion muscles in the arms; ankle dorsiflexion and toe dorsiflexion muscles in the legs) according to UK Medical Research Council (MRC) criteria (Hattori et al., 1999). Impairment was assessed for various sensory modalities (vibration, joint position, pain and light touch) as mild, moderate or severe. Associated findings, including deafness, pupillary abnormalities, scoliosis and dementia, were also assessed. Routine blood chemistry results were reviewed, as were those of CSF analysis and cranial and limb MRI. Detailed family histories were obtained.

Ability to carry out activities of daily living was evaluated according to the modified Rankin scale as follows: 0, normal; 1, non-disabling symptoms not interfering with lifestyle; 2, minor disability from symptoms leading to some restrictions of lifestyle but not interfering with patients’ capacity to look after themselves; 3, moderate disability from symptoms that significantly interfered with lifestyle or prevented fully independent existence; 4, moderately severe disability from symptoms that clearly precluded independent existence, although patients did not need constant attention day and night; 5, severe disability involving total dependence, including constant care day and night.

**Electrophysiological analysis**

Nerve conduction was assessed for the median, ulnar, tibial and sural nerves. Recordings were performed by standard methods using surface stimulating and recording electrodes (Hattori et al., 1999; Misu et al., 1999, 2000; Koike et al., 2001). Motor nerve conduction velocity (MCV), distal motor latency (DL) and compound muscle action potential (CMAP) were recorded for the median, ulnar and tibial nerves. Sensory nerve conduction velocity (SCV) and sensory nerve action potential (SNAP) were assessed for the median and sural nerves.
Pathological study
Sural nerve biopsy was performed in 44 patients. Specimens were processed for glutaraldehyde-fixed, epoxy resin-embedded semithin sections and formalin-fixed, paraffin-embedded sections. Electron microscopic observations and teased-fibre studies were also performed in some cases. The morphometric pathological assessment of all nerve biopsy specimens was performed at the Department of Neurology, Nagoya University Graduate School of Medicine, according to the previously described methods (Sobue et al., 1990; Misu et al., 1999; Hattori et al., 1999). Sural nerve biopsy was essentially as described previously (Sobue et al., 1990; Hattori et al., 1999; Misu et al., 1999). Specimens were fixed in 2% glutaraldehyde in 0.025 M cacodylate buffer at pH 7.4, and processed for semithin, ultrathin or teased-fibre studies. Density of myelinated fibres was assessed directly from the toluidine blue-stained semithin transverse sections of sural nerves using a computer-assisted image analyser (Luzex FS; Nikon, Tokyo, Japan) as described previously (Sobue et al., 1989, 1990, 1997). Unmyelinated fibre density was assessed using the same system, from electron microscopic photographs at ×10 000 magnification, taken to randomly cover uranyl acetate-stained ultrathin transverse sections (Sobue et al., 1989; Hattori et al., 1999). Isolated single nerves were prepared for teased-fibre analysis, in which the pathological condition of each fibre was evaluated according to criteria described previously (Sobue et al., 1989; Dyck et al., 1993). Part of the biopsy specimen was fixed in 10% buffered formalin solution, embedded in paraffin, and observed using haematoxylin and eosin staining as well as Klüver-Barrera staining.

Axonal and demyelinating features of sural nerve pathology were assessed as follows. We took axonal loss, axonal sprouts and axonal pathology in the teased-fibre preparations as axonal histopathological features. Clusters of two or more small myelinated fibres surrounded by basal lamina were designated as axonal sprouts, as described previously (Koike et al., 2001; Vital et al., 2001). Such sprouting is considered a marker of axonal neuropathy (Dyck et al., 1993; De Jonghe et al., 1999; Misu et al., 2000; Vital et al., 2001). Hypertrophic changes of nerve fascicles, onion bulbs, tomacula or globule formation and demyelinating pathology in teased nerve fibre preparations were taken as demyelinating features (Sobue et al., 1990, 1997; Dyck et al., 1993; Sander et al., 1998, 2000).

Statistical analysis
Clinical, electrophysiological and pathological data were compared using the $\chi^2$ test and the Mann–Whitney U test. ANOVA (analysis of variance) with a two-way Student t-test was used for continuous variables with a normal distribution. Correlation studies were performed using Spearman’s regression analysis. Statistical significance was considered to exist when the $P$ value < 0.05.

Results
Patients with PMP22 duplication, MPZ mutations and Cx32 mutations
Clinical features of 205 CMT patients are summarized in Table 1. The mean age at examination, age at onset and duration of illness were essentially similar in the three groups with PMP22 duplication, MPZ mutations and Cx32 mutations. An age of onset <10 years was most frequent for all mutations, but the distribution pattern of age at onset was different among the three gene abnormalities. A gradual unimodal decrease with advancing age was seen for PMP22 duplication and Cx32 mutations, and a rather diffuse distribution among all ages was seen for MPZ mutations (Fig. 1). The male-to-female ratio was nearly equal for PMP22 duplication and Cx32 mutations, and a rather diffuse distribution among all ages was seen for MPZ mutations (Fig. 1). The male-to-female ratio was nearly equal for PMP22 duplication and MPZ mutations; for Cx32 mutations, only male patients were selected. Muscle weakness and atrophy, predominantly in the lower legs in a distally accentuated pattern, was common to PMP22 duplication, MPZ mutations and Cx32 mutations. Muscle hypertrophy,
particularly in the calf muscle, was seen in five patients with PMP22 duplication and three patients with MPZ mutations. Sensory impairment for all modalities was pronounced in the distal legs, and areflexia mainly in the lower legs was common in all three gene abnormalities. Associated symptoms of deafness, pupillary abnormality and scoliosis, which were present for all three gene abnormalities, were more frequent for MPZ and Cx32 mutations. Serum creatine kinase (CK) elevation was also more frequently observed for MPZ mutations than for PMP22 duplication. CSF protein elevations were present in 38–76% of patients, particularly in the subgroup with median nerve MCV >38 m/s (Table 2). In MPZ mutations, the distribution of median MCVs was also unimodal, peaking at 21.1 m/s with a range of 22.8–46.6 m/s (Fig. 2). In Cx32 mutations, the median nerve MCVs were distributed in a bimodal pattern, representing two subgroups with MCV either >38 m/s or ≤38 m/s (Fig. 2). Distal latencies were significantly less prolonged, and CMAPs and SNAPs were markedly reduced in all nerves, particularly in the lower legs, for all three gene abnormalities (Table 2). The shape and duration of proximal and distal CMAPs were similar, demonstrating absence of conduction block or temporal dispersion in all three gene abnormalities. In PMP22 duplication, the median nerve MCVs were markedly reduced below 38 m/s, and the distribution of MCVs was unimodal, peaking at 21.1 m/s (Fig. 2). In Cx32 mutations, the distribution of median MCVs was also unimodal, peaking at 33.2 m/s with a range of 22.8–46.6 m/s (Fig. 2). In MPZ mutations, the median nerve MCVs were distributed in a bimodal pattern, representing two subgroups with MCV either >38 m/s or ≤38 m/s (Fig. 2). Distal latencies were significantly less prolonged, and CMAPs and SNAPs were significantly more decreased in the subgroup with median nerve MCV >38 m/s (Table 2). These findings suggested two distinctive subgroups of MPZ mutations with either demyelinating or axonal phenotypes: MCV >38 m/s, showing an axonal phenotype, and MCV ≤38 m/s, showing a demyelinating phenotype. Several clinical features differed between the above-mentioned MPZ mutation subgroups (Table 1). Patients with MCV >38 m/s were older at onset than those with MCV ≤38 m/s, and in the former group family neuropathic sign was often not apparent. Associated symptoms of sensorineural deafness and pupillary abnormality (Adie’s pupil) were more frequent in those with MPZ mutations than for PMP22 duplication. CSF protein elevations were present in 38–76% of patients, particularly in the subgroup with MCV >38 m/s (Table 2). In MPZ mutations, the distribution of median MCVs was also unimodal, peaking at 21.1 m/s with a range of 22.8–46.6 m/s (Fig. 2). In Cx32 mutations, the median nerve MCVs were distributed in a bimodal pattern, representing two subgroups with MCV either >38 m/s or ≤38 m/s (Fig. 2). Distal latencies were significantly less prolonged, and CMAPs and SNAPs were significantly more decreased in the subgroup with median nerve MCV >38 m/s (Table 2). These findings suggested two distinctive subgroups of MPZ mutations with either demyelinating or axonal phenotypes: MCV >38 m/s, showing an axonal phenotype, and MCV ≤38 m/s, showing a demyelinating phenotype. Several clinical features differed between the above-mentioned MPZ mutation subgroups (Table 1). Patients with MCV >38 m/s were older at onset than those with MCV ≤38 m/s, and in the former group family neuropathic sign was often not apparent. Associated symptoms of sensorineural deafness and pupillary abnormality (Adie’s pupil) were more frequent in those with MPZ mutations than for PMP22 duplication. CSF protein elevations were present in 38–76% of patients, particularly in the subgroup with MCV >38 m/s (Table 2). In MPZ mutations, the distribution of median MCVs was also unimodal, peaking at 21.1 m/s with a range of 22.8–46.6 m/s (Fig. 2). In Cx32 mutations, the median nerve MCVs were distributed in a bimodal pattern, representing two subgroups with MCV either >38 m/s or ≤38 m/s (Fig. 2). Distal latencies were significantly less prolonged, and CMAPs and SNAPs were significantly more decreased in the subgroup with median nerve MCV >38 m/s (Table 2). These findings suggested two distinctive subgroups of MPZ mutations with either demyelinating or axonal phenotypes: MCV >38 m/s, showing an axonal phenotype, and MCV ≤38 m/s, showing a demyelinating phenotype. Several clinical features differed between the above-mentioned MPZ mutation subgroups (Table 1). Patients with MCV >38 m/s were older at onset than those with MCV ≤38 m/s, and in the former group family neuropathic sign was often not apparent. Associated symptoms of sensorineural deafness and pupillary abnormality (Adie’s pupil) were more frequent in patients in the former, axonal subgroup than in the latter, demyelinating subgroup. Considerable serum CK elevation

### Electrophysiological findings

Mean MCVs in the median and tibial nerves and mean SCVs in the median and sural nerves were uniformly reduced in all three mutations, except for a subgroup of patients with MPZ mutations (Table 2). Distal latency was markedly prolonged for PMP22 duplication and the subgroup with MPZ mutations, but less markedly prolonged for Cx32 mutations and other subgroups with MPZ mutations (Table 2). Mean

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<tr>
<th>Mutations</th>
<th>MCV m/s</th>
<th>DL ms</th>
<th>CMAP mV</th>
<th>Range</th>
<th>SCV m/s</th>
<th>SNAP (μV)</th>
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<td>PMP22 duplication</td>
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<td>Median (n = 117)</td>
<td>21.1 ± 5.7</td>
<td>10.3 ± 2.6</td>
<td>3.3 ± 2.8</td>
<td>(0–12.3)</td>
<td>21.0 ± 7.3</td>
<td>0.9 ± 4.2</td>
<td>(0–17.0)</td>
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<tr>
<td>Tibial (n = 54)</td>
<td>20.2 ± 6.2</td>
<td>11.2 ± 6.2</td>
<td>1.2 ± 2.0</td>
<td>(0–9.3)</td>
<td>20.4 ± 11.4</td>
<td>0.7 ± 0.2</td>
<td>(0–14.5)</td>
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<td>Sural (n = 83)</td>
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<td>MPZ mutations</td>
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<td>≤38m/s</td>
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<td>Median (n = 28)</td>
<td>16.5 ± 7.0</td>
<td>8.7 ± 3.5</td>
<td>8.1 ± 5.4</td>
<td>(0.8–18.1)</td>
<td>21.8 ± 5.4</td>
<td>2.7 ± 3.7</td>
<td>(0–10.5)</td>
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<td>Tibial (n = 19)</td>
<td>21.7 ± 11.1</td>
<td>15.3 ± 7.4</td>
<td>0.9 ± 1.2</td>
<td>(0–4.0)</td>
<td>26.3 ± 6.1</td>
<td>2.0 ± 2.0</td>
<td>(0–4.2)</td>
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<td>Sural (n = 16)</td>
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<td>Median (n = 17)</td>
<td>44.3 ± 4.7</td>
<td>3.9 ± 1.6</td>
<td>3.3 ± 3.7</td>
<td>(0–11.0)</td>
<td>51.0 ± 5.7</td>
<td>0.8 ± 0.9</td>
<td>(0–2.8)</td>
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<tr>
<td>Tibial (n = 11)</td>
<td>34.8 ± 3.2</td>
<td>7.7 ± 2.5</td>
<td>0.6 ± 0.8</td>
<td>(0–2.3)</td>
<td>51.5 ± 2.1</td>
<td>0.3 ± 0.6</td>
<td>(0–2.2)</td>
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<td>Sural (n = 10)</td>
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<td>Cx32 mutations</td>
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<tr>
<td>Median (n = 42)</td>
<td>33.2 ± 5.7</td>
<td>5.3 ± 1.7</td>
<td>2.0 ± 1.8</td>
<td>(0–8.1)</td>
<td>38.6 ± 6.4</td>
<td>4.4 ± 3.8</td>
<td>(0–14.2)</td>
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<tr>
<td>Tibial (n = 17)</td>
<td>22.3 ± 5.7</td>
<td>6.1 ± 2.5</td>
<td>1.1 ± 1.7</td>
<td>(0–6.0)</td>
<td>36.5 ± 8.6</td>
<td>1.8 ± 3.3</td>
<td>(0–12.0)</td>
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<tr>
<td>Sural (n = 16)</td>
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<tr>
<td>Controls (n = 121–191)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Median (n = 191)</td>
<td>57.8 ± 3.7</td>
<td>3.3 ± 0.4</td>
<td>16.4 ± 5.3</td>
<td>(9.5–23.5)</td>
<td>57.8 ± 4.7</td>
<td>35.2 ± 12.6</td>
<td>(18.3–39.5)</td>
</tr>
<tr>
<td>Tibial (n = 121)</td>
<td>46.9 ± 3.5</td>
<td>4.6 ± 0.8</td>
<td>16.3 ± 5.8</td>
<td>(9.2–24.4)</td>
<td>51.0 ± 5.1</td>
<td>17.3 ± 7.1</td>
<td>(8.9–25.7)</td>
</tr>
<tr>
<td>Sural (n = 133)</td>
<td></td>
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</table>

Data are mean ± standard deviation. Ranges of CMAP and SNAP values are shown in parentheses. Control values are those described previously (Koike et al., 2001; Misu et al., 1999, 2000).

Table 2 Nerve conduction study in 205 CMT patients with PMP22 duplication, MPZ mutations and Cx32 mutations
was also more frequent in the axonal subgroup. Other motor and sensory symptoms did not differ significantly between the two subgroups (Table 1). These associated symptoms were seen in association with several types of MPZ mutations.

Pathology of the sural nerve
In PMP22 duplication, myelinated fibre density was variably reduced, ranging from 183 to 6854/mm² (Table 3, Fig. 3). Unmyelinated fibre density was also variably reduced but relatively preserved, ranging from 16157 to 28160/mm². Fibres showing demyelinating pathology in teased-fibre preparations represented 80.1 ± 17.7% of the total, while axonal pathology was seen in 0.7 ± 2.8%. Tomacula and globule formation, and onion bulbs were significantly greater in the demyelinating subgroup than in the axonal subgroup (P < 0.05; Table 3), while unmyelinated fibre density did not differ significantly between subgroups (Table 3, Fig. 3). The frequencies of fibres with demyelinating pathology, tomacula, and globule formation, and onion bulbs were significantly greater in the demyelinating subgroup (P < 0.0001, P < 0.001 and P < 0.05, respectively). An uncompacted myelin sheath was occasionally observed. In contrast, the frequency of fibres with axonal pathology and the density of axonal sprouts were significantly greater in the axonal subgroup (P < 0.01 and P < 0.05, respectively; Table 3, Fig. 4). In individual patients with MPZ mutations, pathological features in which either demyelinating or axonal involvement was predominant, combined or concomitant demyelinating and axonal pathological findings were rare. Thus, pathological features were distinctive between the demyelinating and axonal subgroups defined by median nerve MCV.

In Cx32 mutations, myelinated fibre density was diminished, but less markedly than in PMP22 duplications and MPZ mutations (Table 3). Unmyelinated fibres were also relatively well preserved (Table 3). Less marked reduction in the myelinated and unmyelinated fibre density reflected the frequent presence of axonal sprouts (Table 3, Fig. 3). Teased-fibre preparations showed fibres with demyelinating and axonal pathologies, but both were mild. Globule and tomacula formation and onion bulb formation were also mild (Table 3, Fig. 4). However, axonal sprouts were frequent, ranging from 340 to 2610/mm² (Table 3). Large myelinated axon loss and axonal sprouts were variable among the patients. Axonal and demyelinating pathologies were invariably present in combination in individual patients, although axonal features were predominant.

Median nerve MCVs, CMAPs and sural nerve pathology in relation to disease advancement
In PMP22 duplication, slowed median MCV (≤38 m/s) was seen for all ages at examination (Fig. 5A) and durations of illness (data not shown). Median nerve CMAPs tended to decrease with advancing age (r = 0.28, P < 0.013; Fig. 5A) and with disease duration (not significant; data not shown). In Cx32 mutations, moderately slowed MCV (22.8–46.6 m/s)
was noted consistently for all ages at examination (Fig. 5A) as well as disease durations (data not shown). Median nerve CMAPs decreased with age ($r = 0.45$, $P < 0.0013$; Fig. 5A), and tended to decrease with duration of illness (not significant; data not shown). In MPZ mutations, however, two subgroups of MCVs were consistently present through all ages at examination (Fig. 5A) and durations of illness (data not shown). No tendency for CMAPs to decrease with advancing age or disease duration was observed (Fig. 5A).

Sural nerve pathology also changed markedly with disease advancement (Fig. 5B). In the PMP22 duplication, myelinated fibre density, in particular that of large myelinated fibres, decreased significantly with increasing age at examination ($r = 0.71$, $P < 0.0001$; Fig. 5B) and duration of illness ($r = 0.40$, $P < 0.001$; data not shown). Onion bulbs were rare in young patients, but their frequency increased with advancing age ($r = 0.42$, $P < 0.01$; Fig. 5B). In Cx32 mutations, large myelinated fibre density was relatively preserved in the younger patients, but decreased markedly with advancing age ($r = 0.81$, $P < 0.001$; Fig. 5B) and duration of illness ($r = 0.37$, $P < 0.05$; data not shown). Axonal sprouts were more frequent in older patients, and the density of axonal sprouts correlated well with age at examination ($r = 0.68$, $P < 0.01$; Fig. 5B) and duration of illness ($r = 0.43$, $P < 0.01$; data not shown). In cases with MPZ mutations, two small but distinctive subgroups were present, and were difficult to assess.

The MCV slowing was consistently preserved in spite of pathological changes in sural nerves in patients with PMP22 duplication and Cx32 mutations (Fig. 6). Median nerve MCVs were consistently slowed independently of large myelinated fibre loss, degree of onion bulb formation and axonal sprouts in the sural nerves (Fig. 6).

The findings indicate that MCVs in these three main genetic groups are consistently preserved independently of age at examination and disease duration; in patients with PMP22 duplication and Cx32 mutations, slowed MCVs are consistently preserved independently of the development of pathological changes. In contrast, CMAPs and large myelinated fibre populations tended to decrease with advancing age and disease duration. In cases with MPZ mutations, two relatively small but distinct subgroups with different MCVs were present, and tendencies related to age, disease duration and pathology were difficult to assess.

**Concordance and discordance in MCVs among siblings, and gene mutations**

In PMP22 duplication, all patients showed MCVs ≤38 m/s, though the extent of MCV reduction was variable. MCVs of
38 m/s were seen concordantly among siblings in the 18 families in whom MCV was examined systemically (Table 4). In MPZ mutations, probands with MCV ≤38 m/s in the median nerve (demyelinating subgroup) showed good concordance, with MCVs ≤38 m/s among siblings in the four families examined. Probands showing a median nerve MCV >38 m/s (axonal subgroup) exhibited good concordance, with MCV >38 m/s being noted among siblings in the four families (Table 4). The nature and position of amino acid substitutions in the MPZ protein were distinctly different among subgroups of patients with demyelinating and axonal phenotypes (Table 4), suggesting that demyelinating and axonal phenotypes in MCV are concordant in the siblings and are closely related to the nature and position of the amino acid substitution in MPZ.

In Cx32 mutations, the probands showed variable median nerve MCVs, including values more and less than 38 m/s. MCVs were discordant in terms of the 38 m/s cut-off value among siblings in the six families examined (Table 4). MCVs in these families were concordant among siblings in showing a range from 22.8 to 46.6 m/s. Thus, in most of the families, median nerve MCVs were discordant at the division point of 38 m/s but were concordant when the MCV range was set between 22.8 and 46.6 m/s (Table 4).

**Muscle wasting, CMAPs and MCVs**

Muscle weakness and atrophy were most pronounced in the distal portion of the leg and were noted to a lesser extent distally in the upper limbs; proximal muscles were only minimally involved. This distal and lower-limb-predominant motor involvement was common to all three main types of gene abnormality (Table 5). Table 6 shows the correlation between CMAPs and the strength of distal limb muscles in patients with PMP22 duplication, MPZ mutations and Cx32 mutations. The amplitude of CMAPs of median, ulnar and tibial nerves correlated significantly with corresponding distal muscle strengths ($r = 0.35–0.69$, $P < 0.05–0.0005$; Table 5).
In contrast, MCVs of median, ulnar and tibial nerves did not correlate with distal muscle strength in patients with PMP22 duplication, MPZ mutations or Cx32 mutations. These findings indicate that weakness in distal limb muscles was a consequence of reduced CMAP amplitude, not the slowing of MCV, in all groups.

**Discussion**

In CMT patients with PMP22 duplication, almost all patients showed predominantly demyelinating features in the nerve conduction study and pathological examination, with variable severity among individuals. MCVs were \( \approx 38 \text{ m/s} \), independently of age and disease duration. However, features of axonal loss, axonal sprouts and axonal changes in teased-fibre preparations as well as the decrease in CMAP amplitude were variably present irrespective of marked slowing of nerve conduction. These observations were in good agreement with previous reports (Kaku et al., 1993a; Thomas et al., 1997; Birouk et al., 1997; Garcia et al., 1998; Krajewski et al., 2000; Dubourg et al., 2001a). Since PMP22 duplication was shared among these patients, variability of demyelinating and axonal pathology between individual patients must be attributed to factors other than PMP22 duplication. Important factors demonstrated in this study were age and disease duration. CMAP reduction, axonal loss and onion bulb formation were more pronounced in advanced disease. Other factors could be the genetic background or environmental differences, as demonstrated in previous reports showing that the degree of reduction of nerve conduction was variable even in a family pedigree and in identical twins (Kaku et al., 1993a, b; Garcia et al., 1995, 1998; Birouk et al., 1997). As reported so far, PMP22 duplication thus induces a mainly demyelinating phenotype, while features of axonal pathology are present concomitantly with advancing disease.

In cases with MPZ mutations, axonal and demyelinating phenotypes were clearly differentiated into two subgroups. Patients in the axonal subgroup rarely showed concomitant demyelinating phenotypes, and patients in the demyelinating subgroup rarely showed axonal features. These axonal or demyelinating phenotypes were also concordant among siblings in individual families. Furthermore, the nature and position of the MPZ gene mutation did not overlap between these two subgroups, indicating that axonal or demyelinating phenotypes are determined mainly by the nature and position of mutations of the MPZ gene. Apart from the MPZ gene mutation, the overall genetic background and environmental factors may have little effect on phenotypic variation in patients with the MPZ mutation. Age- and duration-dependent clinical and pathological changes could also be present, as was seen for PMP22 duplication, but we could not assess these issues because of relatively small numbers of patients in the subgroups.

Frequent association of neural deafness and pupillary abnormality in patients with the axonal phenotype of MPZ mutations was characteristic, confirming previous observations (Chapon et al., 1999; De Jonghe et al., 1999; Misu et al., 2000). Prominent serum CK elevation was also more frequent.
Fig. 5 Scattergrams of median nerve MCVs, CMAPs (A) and sural nerve pathology (B) in relation to age at examination in CMT patients. Closed circles and open circles in the panel labelled ‘MPZ mutations’ indicate patients with median nerve MCVs >38 m/s (corresponding to axonal phenotypes) and ≤38 m/s (corresponding to demyelinating phenotypes), respectively. In A, the dotted lines indicate the maximum and minimum values in each scattergram, and the 38 m/s level.
in patients with MPZ mutation in the axonal subgroup. These associated symptoms were not restricted to specific MPZ mutations, but were widely present among patients with axonal phenotypes. These observations suggest that the mechanism that induces the axonal phenotype may also induce associated symptoms.

In Cx32 mutations, median nerve MCV was moderately slowed within a relatively restricted range of 22.8–46.6 m/s, confirming previous reports (Hahn et al., 1990; Nicholson et al., 1993, 1998; Dubourg et al., 2001a, b). Pathologically, axonal features predominated but demyelinating features were present concomitantly, suggesting a mixture of axonal and demyelinating pathology. These MCVs and pathological phenotypes were independent of age and disease duration. However, other axonal markers, such as decreased CMAP amplitudes, axonal loss and axonal sprouts, were pronounced in patients with advanced disease. Cx32 mutations may induce both axonal and demyelinating features irrespective of the nature and position of the mutation in the Cx32 gene, with median MCVs showing a moderately decreased range.

Strikingly, median nerve MCVs were consistent independently of age, disease duration and pathological alterations in all three groups of genetic abnormality (Table 6). By consensus, the division point for axonal and demyelinating phenotypes of CMT has been considered to be 38 m/s for median nerve MCV (Dyck and Lambert, 1968; Buchthal and Behse, 1977; Harding and Thomas, 1980). Nerve conduction criteria for axonal and demyelinating phenotypes have been verified by observations in PMP22 duplication (Kaku et al., 1993a, b; Nicholson and Nash, 1993; Nicholson et al., 1998; Paraskevas et al., 1998). In this study we confirmed the usefulness of this nerve conduction cut-off value in MPZ mutations. However, in Cx32 mutations, 38 m/s for median nerve MCV was not a useful division point. Median nerve

Fig. 6 Scattergrams of median nerve MCVs in relation to severity of sural nerve pathology [large myelinated fibre density (MFD), density of onion bulbs and axonal sprouts]. Closed circles and open circles in the panel labelled ‘MPZ mutations’ indicate patients with median nerve MCVs >38 m/s (corresponding to axonal phenotypes) and ≤38 m/s (corresponding to demyelinating phenotypes), respectively. Dotted lines indicate the maximum and minimum values in each scattergram.
MCV for Cx32 mutations ranged around 38 m/s but was restricted to a range between 22.8 and 46.6 m/s. The observation that median nerve MCVs are well maintained independently of age, disease duration and clinicopathological changes indicates that median nerve MCV is an excellent marker for genetically determined CMT phenotypes. In contrast, the amplitude of CMAPs, axonal loss (particularly for large axons), the density of axonal sprouts and onion bulb formation changed significantly with age and disease duration (Table 6). Distally accentuated muscular wasting was characteristic of all three genotypes and progressed with advancing age (Dyck et al., 1989; Birouk et al., 1997), showing good correlation with CMAP amplitude in the corresponding muscles. Disease duration, however, correlated less well with these features than did age at examination, suggesting that the disease process may begin early, at perinatal or embryonic stages, as suggested previously (Dyck et al., 1989; Killian et al., 1996; Garcia et al., 1998). Disease duration, which represents the period after the disease process has crossed the clinical threshold, appears to correlate less well with the pathological changes and CMAPs than age at examination. Age-dependent decreases in CMAPs and increases in axonal pathology and clinical disability from neuropathic deficits were in good agreement with previous observations in CMT1 patients (Dyck et al., 1989).

Taking all the results in this study together, we can identify two distinct clinicopathological phenotypic features, one that is independent of disease advancement and the other with changing phenotypic features according to disease advancement. The MCVs and the predominance of demyelinating or axonal phenotypes are included in the former category, and axonal features, such as CMAP amplitude, large-axon loss, axonal sprouts and distally accentuated muscle wasting, are included in the latter category. The present study shows that axonal features that change in relation to disease advance-

### Table 4 Gene mutations and median nerve MCV and concordance and discordance among siblings

<table>
<thead>
<tr>
<th>Gene mutations and median nerve MCV in the proband</th>
<th>Mutations</th>
<th>No. of families; MCV concordance and discordance among siblings in families examined systemically</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Concordance to MCV ≤38 m/s</td>
</tr>
<tr>
<td>PMP22 duplication</td>
<td>Duplication of PMP22 gene</td>
<td>18 (2–5)</td>
</tr>
<tr>
<td>MPZ mutations</td>
<td>Asp35Tyr, Ile62Phe, Ser63del, Tyr68Cys, Gly93Glu, Arg98Cys, Val146Phe</td>
<td>4 (2–4)</td>
</tr>
<tr>
<td>MCV ≤38 m/s</td>
<td>Asp75Val, His81Arg, Thr124Met Lys130Arg, Gly167Arg</td>
<td>0</td>
</tr>
<tr>
<td>MPZ mutations</td>
<td>Ser26Leu, Thr55Ala, Gln57His, Val63Ile, Phe69Leu, Ser128stop, Val139Met, Arg142Gln, Arg142Trp, Pro172Arg, Val177Ala, Arg183His, Thr191Ala, Cys201Tyr, Ala282frameshift</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are number of patients in the family whose median nerve MCV was estimated.

### Table 5 Correlation between CMAP/MCV and muscle strength

<table>
<thead>
<tr>
<th>Mutations/nerves</th>
<th>$n$</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PMP22 duplication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMAP vs distal MS</td>
<td>86</td>
<td>0.35</td>
<td>&lt;0.0009</td>
</tr>
<tr>
<td>MCV vs distal MS</td>
<td>86</td>
<td>0.16</td>
<td>NS</td>
</tr>
<tr>
<td>Ulnar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMAP vs distal MS</td>
<td>63</td>
<td>0.44</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>MCV vs distal MS</td>
<td>63</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Posterior tibialis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMAP vs distal MS</td>
<td>79</td>
<td>0.36</td>
<td>&lt;0.0014</td>
</tr>
<tr>
<td>MCV vs distal MS</td>
<td>79</td>
<td>0.15</td>
<td>NS</td>
</tr>
<tr>
<td><strong>MPZ mutations</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMAP vs distal MS</td>
<td>28</td>
<td>0.36</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MCV vs distal MS</td>
<td>28</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Ulnar</td>
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<tr>
<td>CMAP vs distal MS</td>
<td>17</td>
<td>0.43</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MCV vs distal MS</td>
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<td>0.14</td>
<td>NS</td>
</tr>
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<td>Posterior tibialis</td>
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<td></td>
<td></td>
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<tr>
<td>CMAP vs distal MS</td>
<td>17</td>
<td>0.47</td>
<td>&lt;0.018</td>
</tr>
<tr>
<td>MCV vs distal MS</td>
<td>17</td>
<td>0.17</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Cx32 mutations</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMAP vs distal MS</td>
<td>19</td>
<td>0.69</td>
<td>&lt;0.0032</td>
</tr>
<tr>
<td>MCV vs distal MS</td>
<td>19</td>
<td>0.21</td>
<td>NS</td>
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<tr>
<td>Ulnar</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CMAP vs distal MS</td>
<td>19</td>
<td>0.68</td>
<td>&lt;0.0079</td>
</tr>
<tr>
<td>MCV vs distal MS</td>
<td>19</td>
<td>0.30</td>
<td>NS</td>
</tr>
<tr>
<td>Posterior tibial</td>
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<td></td>
<td></td>
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<tr>
<td>CMAP vs distal MS</td>
<td>19</td>
<td>0.38</td>
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<tr>
<td>MCV vs distal MS</td>
<td>19</td>
<td>0.16</td>
<td>NS</td>
</tr>
</tbody>
</table>

MS = muscle strength assessed as MRC score; distal MS = thenar and finger flexion muscles for the median nerves, interosseous muscles for the ulnar nerves, and triceps surae muscles and toe dorsiflexion muscles for the posterior tibial nerve. MS was assessed as MRC score for the examined muscles and averaged for the examined muscles for each individual.
ment, particularly large-axon loss and decreased CMAP amplitude, constitute a major determinant of clinical manifestations such as muscle weakness and atrophy in all three common genotypic groups of CMT.

The molecular mechanisms that induce axonal involvement as disease advances, which have been demonstrated particularly in PMP22 duplication and Cx32 mutations, are poorly understood, and may differ among mutations. In the case of PMP22 duplication, axonal dysfunction could result from the primary process of demyelination, as suggested in previous studies (Dyck et al., 1989; Garcia et al., 1998; Sancho et al., 1999; Scherer, 1999; Krajewski et al., 2000). Local biochemical changes in axons, such as decreased neurofilament phosphorylation, increased neurofilament density and decreased axonal transport due to demyelinating Schwann cells, could accrue to produce axonal dysfunction and eventual axonal loss (de Waegh and Brady, 1990, de Waegh et al., 1992; Watson et al., 1994; Sahenk et al., 1999). In Cx32 mutations, issues concerning mechanisms of progressive axonal involvement are further complicated, since an axonal phenotype is predominant but demyelinating features are concomitantly present to variable degrees in individual patients. Unknown mechanisms may induce axonal dysfunction and loss directly, while concomitant demyelinating features could also induce axon loss, as is suspected for PMP22 duplication. Studies using in vivo models with specific mutations of Cx32 and different genetic or environmental backgrounds are needed to provide better understanding of the molecular mechanisms.

The distally accentuated muscle wasting characteristic of CMT was strongly correlated with the reduction of the functioning large axonal population, as assessed by CMAPs, and was not correlated with slowing of nerve conduction. This finding was common to patients with PMP22 duplication, MPZ mutations and Cx32 mutations. Similar observations were reported in patients with PMP22 duplication (Krajewski et al., 2000). In that report, muscle wasting and sensory impairment that was accentuated in the distal extremities correlated well with corresponding reductions in CMAPs and SNAPs, but not with slowing of motor and sensory nerve conduction. Our observations are in agreement with these reported observations in PMP22 duplication, while we have demonstrated further that clinical phenotypes of patients with MPZ and Cx32 mutations are also determined by reduction of the functioning large-axon population. Pathological study of autopsy cases of CMT with a hypertrophic form of extensive demyelination and an axonal form with massive axonal sprouting both demonstrated distally accentuated axon loss along peripheral nerve trunks that showed relatively well-preserved axons proximally (Smith et al., 1980; Berciano et al., 1986; Bird et al., 1997). These pathological observations in CMT patients showing distally pronounced axon loss irrespective of the axonal or demyelinating phenotypes agree with the present study in showing distally accentuated muscle wasting in both axonal and demyelinating phenotypes.

In conclusion, our study demonstrates that three myelin-related protein gene abnormalities, PMP22 duplication, MPZ mutations and Cx32 mutations, resulted in a wide variety of demyelinating and axonal features. PMP22 duplication induced demyelinating changes; MPZ mutations induced distinctive subgroups with a demyelinating or axonal phenotype depending on the nature and position of the mutation; and Cx32 mutations induced predominant axonal and lesser demyelinating features essentially simultaneously. These genetically determined axonal and demyelinating phenotypes corresponded well with median nerve MCVs, which remained constant despite disease advancement. In contrast, clinical manifestations of muscle wasting correlated well with decreases in CMAPs and axonal loss, which became more pronounced as disease progressed.

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