Two divergent types of nerve pathology in patients with different $P_0$ mutations in Charcot-Marie-Tooth disease

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**Article abstract**—In seven unrelated patients with a demyelinating motor and sensory neuropathy, we found mutations in exons 2 and 3 of the $P_0$ gene. Morphologic examination of sural nerve biopsy specimens showed a demyelinating process with onion bulb formation in all cases. In four patients, ultrastructural examination demonstrated uncompacted myelin in 23 to 68% of the myelinated fibers, which is in agreement with the widely accepted function of $P_0$ as a homophilic adhesion molecule. Three patients showed normal compact myelin, but morphology was dominated by the abundant occurrence of focally folded myelin. The two divergent pathologic phenotypes exemplify that some mutations act differently on $P_0$ protein formation or function than others, which is probably determined by site and nature of the mutation in the $P_0$ gene.

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Charcot-Marie-Tooth disease type 1 (CMT1), or hereditary motor and sensory neuropathy type I (HMSN type I), is a demyelinating polyneuropathy, with prevailing autosomal dominant inheritance. Most autosomal dominant cases map to chromosome 17 (CMT1A); a minority map to chromosome 1 (CMT1B) or to another unknown autosomal (CMT1C). CMT1A usually results from a duplication of a 1.5-Mb region on chromosome 17p12 encompassing the gene for the peripheral myelin protein PMP22; a few patients show point mutations in this gene (see review in Patel and Lupski1). Recent analysis of CMT1B families did reveal different point mutations in the gene for the major protein of peripheral myelin, protein zero ($P_0$).2-9 In some sporadic patients with a more severe phenotype, formerly known as Dejerine-Sottas disease (DS), de novo $P_0$ mutations have been identified.10,11 Nerve morphology in most CMT1B families is designated as "hypertrophic demyelinating neuropathy." One report described a demyelinating polyneuropathy with an abundant occurrence of focally folded myelin in a father and son with a $P_0$ mutation.12 Hypomyelination with extensive onion bulb formation dominated the pathology in two severely affected de novo mutation cases.10,13,14

We describe, for the first time, two divergent morphologic phenotypes in seven patients with different $P_0$ mutations.15

**Patients and methods.** Mutation screening of $P_0$ exons was performed in seven unrelated patients without a duplication on chromosome 17p12, by single-stranded conformation polymorphism (SSCP) analysis, followed by sequencing of the relevant DNA region, according to previously described protocols.2,3,8,16-18 The reported $P_0$ mutations were not found in 100 unrelated healthy controls. All patients suffered from a moderately to prominently disabling demyelinating motor and sensory neuropathy with marked conduction slowing (table) and had formerly undergone a sural nerve biopsy. None of the patients had experienced pressure palsies. Patients 1, 5, and 6 used wheelchairs from the ages of 35 years, 10 years, and 7 years, respectively. Patient 3 showed a marked deterioration from the age of 10 months onward and died unexpectedly at the age of 22 months.

Data of patients 1, 4, 5, and 7 are included in previous reports.19-21 Patient 2 is a member of family NL-47.3 Sural nerve biopsy specimens were prepared for light and electron microscopic studies and morphometric examinations using previously described methods.20

**Results.** Patient 2, member of a CMT1B family, showed a three-base-pair deletion in the $P_0$ gene, resulting in a deletion of the codon for serine 34.3 In the six other patients we identified a single base change, leading to an amino acid substitution in codons 5, 69, 101, and 106 of the $P_0$ gene (table). Amino acid numbering was started at the first amino acid (He) of the mature $P_0$ protein, after the 29 amino acids of the signal peptide.22 Different codon 69 sub-

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stitions were identified in the patients 3 and 4. The Arg69His substitution of patient 4 was found in two other CMT1B families.8,9 The unrelated patients 5 and 6 showed an identical mutation in codon 101. These six patients were isolated cases; parents of five patients were normal by clinical and electrophysiologic examination and analysis of parental DNA demonstrated that the amino acid substitutions were de novo mutations. For patient 1 neither the parents nor the nine siblings showed clinical signs of CMT. Chromosomal DNA was available from live siblings only and these did not carry the mutation. Therefore, it is likely but not proven that patient 1 also had a de novo mutation. The patients were heterozygous carriers of the mutation, concordant with autosomal dominant inheritance. A child of patient 4, born in 1992, showed at the age of 2 years clumsy motor performances, absent ankle jerks, and a median motor nerve conduction velocity of 21 m/s. DNA investigation revealed the same Arg69His mutation as in the mother.

Light microscopic examination showed in all cases a chronic demyelinating process with onion bulb formation. Myelinated fiber density was markedly decreased and myelinated fiber diameter histogram showed a preferential loss of large-diameter fibers. Total transverse fascicular area was moderately increased.

Ultrastructural examination in patients 1, 2, 3, and 4 revealed the presence of uncompacted myelin in 23 to 68% of the myelinated fibers. The uncompact ed structure was commonly seen in the inner layers of the myelin sheath, but occasionally it was seen in the outer lamellae as well (figure 1A). At the transitional zone between compacted and uncompacted myelin, the major dense lines split to enclose thin layers of Schwann cell cytoplasm, sometimes with adherens junctions22 (figure 1B). Usually, the intraperiod distance was slightly broadened and sometimes partial fusion of the extracellular membranes occurred over a short distance (figure 1C). Schmidt-Lanterman incisures were frequent and abnormally wide. Occasionally, they could not be distinguished from areas of uncompacted myelin. In patients 1 and 4 several fibers showed undulating major dense lines with dilatation of intraperiod lines only (figure 1D). The compacted parts of the sheaths showed a normal periodicity of myelin. Myelin-like figures were often seen between the uncompacted lamellae, suggestive of incipient myelin degradation (see figure 1B). Several demyelinated fibers were present; tomacula occurred sporadically in the patients 1 to 4. Onion bulb formations occurred rather frequently in the older patients and were composed of thin Schwann cell lamellae. The myelin sheath of nearly all fibers was too thin for the axon diameter, witness the high g-ratios (axon diameter: fiber diameter) (see table).

In patients 5, 6, and 7 the occurrence of many folded myelin loops (tomacula) dominated the pathology (figure 2). In longitudinal sections excessive folded myelin appeared to pass into thin myelin sheaths within the same internode. Fibers outside tomacula were frequently demyelinated or thinly (re)myelinated. Onion bulbs were composed of thin Schwann cell layers and contained many double basement membranes, especially in case 7. Uncompacted myelin was encountered only in a few fibers. Involvement of unmyelinated axons was not obvious in either morphologic type.

### Discussion

We investigated nerve pathology in seven patients with different mutations in the P0 gene. The clinical phenotypes ranged from a severe, infantile neuropathy with very low conduction velocities to a milder neuropathy with later onset. The severe, infantile cases might be classified as DS disease, although they do not show an autosomal recessive inheritance, which is part of the original concept.24 In the future, it would be preferable to classify the inherited neuropathies not by clinical and elec-

### Table: Clinical, electrophysiologic, and morphologic data of patients with different P0 mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>P0 mutation</th>
<th>Age at independent walking</th>
<th>Median MNCV (m/s)</th>
<th>Age at biopsy</th>
<th>MF density in % of normal†</th>
<th>TTFA in % of normal‡</th>
<th>Demyelinated fibers per 100 MF</th>
<th>Onion bulbs per 100 MF</th>
<th>% MF + UCM</th>
<th>% teased MF + tomacula</th>
<th>Mean g-ratio of 100 MF§</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thr51Le</td>
<td>3 y</td>
<td>12</td>
<td>35 y</td>
<td>12</td>
<td>276</td>
<td>9</td>
<td>50</td>
<td>60</td>
<td>&lt;1</td>
<td>0.72</td>
</tr>
<tr>
<td>2</td>
<td>Ser34</td>
<td>normal</td>
<td>12</td>
<td>31 y</td>
<td>14</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>Arg69Cys</td>
<td>no</td>
<td>8</td>
<td>31 y</td>
<td>14</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.75</td>
</tr>
<tr>
<td>4</td>
<td>Arg69His</td>
<td>1 y 10 m</td>
<td>19</td>
<td>12 y</td>
<td>12</td>
<td>230</td>
<td>6</td>
<td>57</td>
<td>60</td>
<td>3 y</td>
<td>0.83</td>
</tr>
<tr>
<td>5</td>
<td>Lys101Arg</td>
<td>2.5 y</td>
<td>11</td>
<td>3 y</td>
<td>3 y</td>
<td>159</td>
<td>6</td>
<td>60</td>
<td>38</td>
<td>2 y</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>Lys101Arg</td>
<td>2.5 y</td>
<td>10</td>
<td>3 y</td>
<td>3 y</td>
<td>139</td>
<td>6</td>
<td>60</td>
<td>45</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>Ile106Leu</td>
<td>2.5 y</td>
<td>7</td>
<td>2 y</td>
<td>2 y</td>
<td>180</td>
<td>6</td>
<td>60</td>
<td>21</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

† Mean MNCV.
‡ Mean density of age-matched controls in number/mm²: 15420 (1—2 y, n = 6); 14170 (2—5 y, n = 6); 10530 (11—20 y, n = 5); 10210 (21—40 y, n = 9).
§ Mean TTFA of age-matched controls in mm*: 0.47 (1—2 y, n = 4); 0.61 (2—5 y, n = 4); 0.87 (11—30 y, n = 5); 0.93 (31—70 y, n = 5).
§ Mean density of age-matched controls in number/mm²: 15420 (1—2 y, n = 6); 14170 (2—5 y, n = 6); 10530 (11—20 y, n = 5); 10210 (21—40 y, n = 9).
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Figure 1. Electron micrographs of myelinated fibers in patients with uncompacted myelin (patients 1-4). (a) Uncompacted lamina of the inner myelin layer. Bar = 500 nm. (b) Uncompacted myelin at the inner and outer parts of the inner myelin layer (open arrow Bar = 500 nm). (c) Myelin figure in uncompacted part of myelin. Bar = 500 nm.
References

In experimental animals, a significant decrease in P4 levels is associated with the maintenance of the normal program of development and function of the hypothalamus-pituitary-gonadal axis, which may be due to a decrease in the activity of the gonadotropins. The suppression of P4 in vivo and in vitro is a result of its action on the pituitary gland, which is essential for the normal function of the pituitary gland. The suppression of P4 may be due to a decrease in the activity of the gonadotropins, which is essential for the normal function of the pituitary gland.


41. Mariman ECM, Gabreëls-Festen AAWM, van Beersum SEC, et al. Prevalence of the 1.5-Mb 17p deletion in families with the major peripheral myelin protein P0 gene show that both proteins contribute to the formation of the major dense line in peripheral nerve myelin. J Neurosci 1995;15:4488–4498.