Charcot-Marie-Tooth disease in northern Sweden: pedigree analysis and the presence of the duplication in chromosome 17p11.2

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Abstract
Sixty-seven patients in 29 families with the diagnosis of Charcot-Marie-Tooth disease or hereditary motor and sensory neuropathy in northern Sweden were examined by pedigree and DNA analysis for the CMT1a duplication within chromosome 17p11.2. There were 39 patients in nine families with Charcot-Marie-Tooth type I and autosomal dominant inheritance and in all these cases the duplication was seen. In six patients in three families with Charcot-Marie-Tooth type I the pedigrees strongly suggested autosomal recessive inheritance. In two patients DNA analysis was not informative but in the others no duplication was shown. There were also 11 "sporadic" patients and one pair of sibs classified as Charcot-Marie-Tooth type I, but there was no duplication shown although in four patients DNA analysis was not informative. In nine patients with Charcot-Marie-Tooth type 2 from five families and in 13 unaffected relatives of Charcot-Marie-Tooth patients the CMT1a duplication was not found.

The finding that CMT patients could be roughly divided into two groups by nerve conduction velocity (NCV) was confirmed, and an approximate dividing value regarding nerve conduction velocity in the motor median nerve in a large sample was 38 m/sec with some overlap. However, there are other studies suggesting a division into three types. For a firm diagnosis of CMT type I cases in autosomal dominant families to be used for linkage analysis, median nerve conduction velocity \( \leq 30 \) m/sec has been required. HMSN I is the most prevalent of all inherited neuromuscular disorders. The majority of families, classified as HMSN Ia (CMT1a), showing linkage to the Duffy blood group locus on chromosome 1, but the majority of families, classified as HMSN Ia and II were the prevalence rate was 16-2 per 100 000. Genetic studies have indicated heterogeneity within HMSN I with a few families classified as HMSN II (CMT1b) showing linkage to the Duffy blood group locus on chromosome 1, but the majority of families, classified as HMSN Ia (CMT1a), showing linkage to markers on chromosome 17. Recently a large duplication of DNA in the proximal short arm of chromosome 17 was shown in cases of CMT1a and a candidate gene for CMT1a, peripher myelin gene PMP-22, was identified mapping in the middle of the duplication.

CMT is usually autosomal dominantly inherited. In most studies sporadic cases are found but with thorough clinical and electrophysiological examination of family members these cases often turn out to be hereditary. There have been a few cases reported classified as HMSN I and HMSN II of probable autosomal recessive inheritance and, as for the recessive type I cases, a tendency towards being clinically more severely affected was observed. However, it was recently found that in nine out of 10 sporadic cases of HMSN I the duplication in chromosome 17 was present as a de novo mutation. Furthermore an X linked dominant form of CMT has been reported and also an X linked recessive form where linkage results indicated at least two genetic loci. In northern Sweden quite a high proportion of sporadic CMT cases was found.

The aim of this study was to examine a series of CMT families and sporadic cases in northern Sweden with pedigree analysis and to estimate the frequency of the CMT1a duplication within chromosome 17p11.2.
Material
A total of 67 patients from 29 families in northern Sweden with the diagnosis of CMT were examined using family history and pedigree analysis and DNA analysis for CMT1a duplication within 17p11.2. The pedigrees are shown in figs 1 to 4. Thirteen unaffected relatives were also examined by DNA analysis for the duplication. The diagnosis of CMT was established in patients with a progressive, unremitting, predominantly motor neuropathy with a typical distal localisation. Other causes of peripheral neuropathy were excluded by history and medical records. Patients with peripheral neuropathy and additional neurological signs, such as extensor plantar response, proximal muscle involvement, or cerebellar ataxia, were excluded. Patients were classified as CMT type 1 or 2 on the basis of nerve conduction velocity (NCV) in the motor and sensory nerve with a separating value of 38 m/sec. One patient in an autosomal dominant CMT1 family (pedigree 2) had NCV 39 m/sec in the motor nerve and was classified as having CMT1. In a pair of equally disabled sibs without a family history, the brother had NCV 29 m/sec and his sister 42 m/sec (sensory NCV in the median nerve was 35 and 39 m/sec respectively) and, although difficult to classify, they were eventually both classified as having CMT1 (pedigree 5). In a few patients NCV was not obtainable and in these cases previous NCV recordings were used for classification.

NCV greater than 38 m/sec, electromyography showing denervation signs, and a typical clinical picture with onset in childhood or adolescence or an evident family history were required for inclusion.

DNA analysis
DNA was extracted from frozen whole blood using phenol-chloroform extraction, digested, hybridised, and separated with electrophoresis according to standard procedures.25

Methods
DNA PROBES
The DNA probes used recognise only single copy fragments and were pVAW409R3a (D17S122), pEW401HE (D17S61), pVAW412R3HEb (D17S125), and pVAW412R3HEc (D17S125). The polymorphic MspI bands with probe pVAW409R3a (D17S122) were 2.8, 2.7, and 1.9 kb, and with probe pEW401HE (D17S61) 5.5 and 4.4 kb. Probe 412 recognises two polymorphisms: pVAW412R3HEb (D17S125) with 10.5 and 5.4 and pVAW412R3HEc (D17S125) with 2.6 and 1.9 kb.12 The inserts were labelled with 1 mCi/ml 32P-dATP or 3P-dCTP (Amersham, 3000 Ci/mM) or both by oligonucleotide labelling. Fifty one samples were analysed in Antwerp with all four probes, 42 samples in Umeå with probe 409, and 12 samples in both Antwerp with all probes and Umeå with probe 409 (tables 1–3).

DNA ANALYSIS
DNA was extracted from frozen whole blood using phenol-chloroform extraction, digested, hybridised, and separated with electrophoresis according to standard procedures.

Figure 1 Pedigree of a family with Charcot-Marie-Tooth disease type 1 (HMSN 1) and autosomal dominant inheritance.
Results
There were 39 cases of CMT1 in nine families with presumed autosomal dominant inheritance in pedigrees 1, 2, 6, 7, 12, 13, 20, 30, and 37 (table 1). Mean age at examination was 36.4 years (8-73 years) and mean NCV, when obtainable in this study, was 23.9 m/sec (16-39 m/sec). In all 39 patients the CMT1a duplication in chromosome 17p11.2 could be shown (table 1).

In three CMT1 families (pedigrees 4, 9, and 31) with six affected cases, the pedigree anal-
lysis indicated autosomal recessive inheritance with consanguinity in families 9 and 31 but not in family 4. In these families, four affected patients did not show the CMT1a duplication but in two patients DNA analysis was not informative (table 2). Mean age at examination was 17.2 years (8–32 years) and mean NCV was 27.0 m/sec (21–36 m/sec).

There were also six "sporadic" CMT1 cases (pedigrees 18, 19, 35, 36, 44, and 45) with no family history of neurological disease and where both parents were examined clinically and electrophysiologically and found not to be affected. Mean NVC was 25.3 m/sec (21–32 m/sec) in the motor median nerve. In one case the DNA analysis was not informative but the
other patients did not show the CMT1a duplication (table 2).

A further five CMT type 1 "sporadic" cases (pedigrees 14, 21, 22, 24, and 25) and one brother and sister pair (pedigree 5) had no previous family history of neurological disease, but relatives were not available for examination. In one patient the analysis was not informative and in the other four no CMT1a duplication was shown (table 2).

Nine CMT type 2 patients were also analysed for the CMT1a duplication. There were six cases in families 3 and 8 with presumed autosomal dominant inheritance and three "sporadic" cases in pedigrees 16, 17, and 23. In three patients the DNA analysis was not informative and in the other cases there was no duplication (table 3). The DNA of 13 clinically and electrophysiologically unaffected relatives was also analysed and no duplication was detected (table 3).

**Discussion**

In all 39 examined cases of CMT1 in nine autosomal dominant families the CMT1a duplication in chromosome 17p11.2 was shown and these patients could accordingly be classified as CMT1a (HMSN IA).

In northern Sweden there seems to be a rather large proportion of isolated or sporadic CMT cases. There are published reports on autosomal recessive CMT1 cases but this form has been considered rare. When recently, in a series of 10 sporadic cases of CMT1, the DNA was analysed for the CMT1a duplication, it was shown that nine out of 10 cases were duplicated following a de novo mutation. However, in the present material from northern Sweden none of the sporadic CMT1 cases or the CMT1 sibs with unaffected parents showed the CMT1a duplication, and for six of these patients the pedigrees strongly suggested autosomal recessive inheritance (pedigrees 4, 9, and 31). In six of the patients the DNA analysis was not informative.

Although paternity was not tested, the re-
Table 2 Charcot-Marie-Tooth patients classified as autosomal recessive (rec) or sporadic (spor) cases. NCV = NCV in the motor median nerve (m/sec). U409 was analyzed in Umeå, A409 and the other probes in Antwerp.

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Sex</th>
<th>Age</th>
<th>NCV (m/sec)</th>
<th>CMT type</th>
<th>A409 U409</th>
<th>412b</th>
<th>412c</th>
<th>401</th>
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<tr>
<td>9</td>
<td>VII.1 F</td>
<td>11</td>
<td>21</td>
<td>CMT1 rec</td>
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<tr>
<td>9</td>
<td>VII.1 M</td>
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<td>CMT1 rec</td>
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<td>4</td>
<td>IV.1 F</td>
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<td>28</td>
<td>CMT1 rec</td>
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<td>4</td>
<td>IV.1 M</td>
<td>17</td>
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<td>4</td>
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<tr>
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<td>IV.4 F</td>
<td>32</td>
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<tr>
<td>11</td>
<td>II.1 M</td>
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<td>29</td>
<td>CMT1 spor</td>
<td>H</td>
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<td>22</td>
<td>II.3 M</td>
<td>20</td>
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<td>CMT1 spor</td>
<td>H</td>
<td>12</td>
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<td>H</td>
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<tr>
<td>25</td>
<td>IV.1 M</td>
<td>42</td>
<td>22</td>
<td>CMT1 spor</td>
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<td>12</td>
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<td>5</td>
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<td>CMT1</td>
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*Not measured in this study; classification based on previous recording. H = homozygote.

The results remain significant for the absence of the CMT1a duplication in our sporadic CMT1 patients, even if a non-paternity rate of 10% is assumed. There was no significant difference in the mean NCV values between the duplicated CMT cases and the non-duplicated sporadic cases of CMT1. For the male sporadic CMT1 patients it is not possible to rule out an X linked mode of inheritance; however, there is no pedigree support for this in our series. It has been a matter of discussion whether proven recessive HMSN I cases (hypertrophic form of Charcot-Marie-Tooth disease) should be classified as HMSN III or HMSN 1 with autosomal recessive inheritance.2324 In this study there was a 24 year old woman (V.2 in pedigree 2) who at the age of 4 years already had an extremely reduced NCV (NCV<10 m/sec) and who clinically showed an early and pronounced peripheral neuropathy. Nerve biopsy showed "a picture compatible with HMSN III". In adolescence the clinical and electrophysiological features of Turner syndrome appeared and she was found to have the karyo-type 46,X (iso Xq). However, she was then shown to belong to an autosomal dominant CMT1 family and the CMT1a duplication in 17p11.2 was found. No other patient in this sample had an age of onset within the first two years of life and in no case where NCV was measured was it 12 m/sec or less. We suggest that most of the sporadic CMT1 patients and sibships without the CMT1a duplication in this sample from northern Sweden are autosomal recessive CMT1 cases.

The finding of a duplication in chromosome 17p11.2 in CMT1a has considerably facilitated the examination of patients with peripheral neuropathy. When the duplication is found the diagnosis of CMT1a is established and reliable genetic counselling and prenatal diagnosis can be offered. However, sometimes the CMT1a duplication may be difficult to show using Southern blot hybridisation of MspI digested DNA with the polymorphic probes 409, 412, and 410 owing to homozgyosity. In these cases the use of additional probes, or of a different method such as quantitative dosage analysis, is needed to show the presence or absence of the CMT1a duplication. In this study 34 of 39 cases in the CMT1 autosomal dominant families were informative for the CMT1a duplication using only probe 409, and when using all four probes all 39 cases were informative.

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