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 1 and autosomal dominant inheritance and in all these cases the duplication was seen. In six patients in three families with Charcot-Marie-Tooth type 1 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 1, 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.

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Charcot-Marie-Tooth disease (CMT) or peroneal muscular atrophy or hereditary motor and sensory neuropathies (HMSN) is a genetically heterogeneous group of disorders characterised by slowly progressive motor and sensory neuropathy with foot and sometimes hand deformities and onset usually in childhood.

Current classification is based on the classification by Dyck and Lambert using clinical, genetic, electrophysiological, and pathological criteria.¹ HMSN types I and II (CMT1 and 2), although clinically difficult to separate, were divided into the hypertrophic de- and remyelinating form with severely reduced nerve conduction velocities (HMSN I) and the axonal, neuronal form with normal or moderately reduced nerve conduction velocities (HMSN II). Both HMSN I and II were autosomal dominantly inherited. HMSN type III was described as a hypertrophic neuropathy with autosomal recessive inheritance and corresponded to Dejerine-Sottas disease. A motor form of CMT without any sensory impairment can also be distinguished, known as hereditary distal spinal muscular atrophy.

The finding that CMT patients could be roughly divided into two groups by nerve conduction velocity (NCV) was confirmed,² 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.³ However, there are other studies suggesting a division into three types.^{4,5} For a firm diagnosis of CMT type 1 cases in autosomal dominant families to be used for linkage analysis, median nerve conduction velocity ≤ 30 m/sec has been required.⁶

HMSN I is the most prevalent of all inherited neuromuscular disorders.⁷ In the two northernmost provinces of Sweden the prevalence of all types of CMT was estimated to be 20.1 per 100 000 (in total 104 cases) and for CMT type 1 the prevalence rate was 16.2 per 100 000.⁸

Genetic studies have indicated heterogeneity within HMSN I with a few families classified as HMSN IB (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.^{9,10} 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, peripheral myelin gene PMP-22, was identified mapping in the middle of the duplication.¹¹⁻¹⁸

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.¹⁹ 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.²⁰ 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.²¹ 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.²²⁻²⁴ In northern Sweden quite a high proportion of sporadic CMT cases was found.⁸

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 median 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 median 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. Needle electromyography (EMG) was performed in most of the autosomal dominant CMT1 cases, in all CMT2 cases, and in all sporadic cases. For the diagnosis of CMT2,

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. Paternity was not tested in the sporadic CMT cases.

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.^{12,13} The inserts were labelled with 1 mCi/ml ³²P-dATP or ³²P-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.²⁵

Figure 1 Pedigree of a family with Charcot-Marie-Tooth disease type 1 (HMSN I) and autosomal dominant inheritance.

Figure 2 Pedigrees of Charcot-Marie-Tooth type 1 families with probable 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 ana-

Figure 3 Three Charcot-Marie-Tooth type 1 families where pedigrees indicate autosomal recessive inheritance and pedigrees from 12 sporadic cases.

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

Figure 4 Pedigrees from patients with Charcot-Marie-Tooth disease type 2 (HMSN II).

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.⁸ There are published reports on autosomal recessive CMT1 cases but this form has been considered rare.²⁰ 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.²¹ 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 1 Charcot-Marie-Tooth type 1 families with autosomal dominant inheritance. NCV=NCV in the motor median nerve (m/sec). U409 was analysed in Umeå, A409 and the other probes were analysed in Antwerp

Pedigree		Age	NCV (m/sec)	A409	U409	412b	412c	401
1	III.4	62	32	112		122	112	H
1	IV.7	37	29	122	122			
1	III.6	52	23	112		122	112	H
1	IV.8	20	20	122		122	112	H
1	IV.9	32	37	122		122	112	H
2	VI.3	20	21		122			
2	V.21	42	23	112	112	122	112	H
2	IV.22	56	21	112		122	112	H
2	V.20	32	21	112		112	122	H
2	VI.2	8		122		122	112	H
2	V.2	23	7*	112				H
2	IV.5	51	25	122		122	112	H
2	IV.18	44	20	122	122	122	112	H
2	V.13	14	18		122			
2	IV.21	34	25		122			
2	IV.19	42	19		122			
2	IV.25	65			112			
6	III.5	30	23	H		H	H	122
6	II.4	66	22	223		H	H	122
6	III.6	22	18	H		H	H	122
7	II.2	57	23	112		H	H	122
7	III.5	21	20	112		H	H	112
7	III.3	28	28	112		H	H	122
12	II.6	61	19	122		122	112	H
12	III.15	27	23	122		122	112	H
12	III.14	37	22	H	H	122	122	H
12	III.13	40	24	122		122	122	H
12	IV.2	17	18	H		112	122	H
12	IV.3	14	39	H		122	112	H
13	II.1	73	22	112	122	H	H	H
13	III.5	44	28	122		H	H	H
13	III.2	51	24	122	122	H	H	H
13	III.6	41	27	122	122	H	H	H
20	II.2	44	33	122	122			H
30	III.3	31	30	112		122	112	H
30	IV.2	10	30	112		122	112	H
37	II.2	47	16	112	112		?	122
37	III.1	14	21	112	112	H	H	H
37	III.2	12	17	112	112	H	H	H

* Not measured in this study; classification based on previous recording.
H = homozygote.

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 analysed in Umeå, A409 and the other probes in Antwerp

Pedigree	Sex	Age	NCV (m/sec)	CMT type	A409	U409	412b	412c	401
9	VII.1	F	11	21	CMT 1 rec?	H		H	12
9	VII.2	M	8	21	CMT 1 rec?	H		H	12
4	IV.1	F	20	28	CMT 1 rec?		12		
4	IV.2	M	17	36	CMT 1 rec?	H		H	H
4	IV.6	M	15	29	CMT 1 rec?		12		
31	IV.4	F	32	23*	CMT 1 rec?	H	12	H	H
18	II.2	M	12	29	CMT 1 spor	H		12	H
19	II.4	M	15	32	CMT 1 spor		22		
35		M	21	28	CMT 1 spor		12		
36		F	17	23	CMT 1 spor		12		
44		F	23	22	CMT 1 spor		12		
45		M	24	21	CMT 1 spor		12		
14	II.3	M	40	23	CMT 1 spor		12		
22		M	19	28	CMT 1 spor		12		
25		M	38	21*	CMT 1 spor	12	12	H	H
21	II.4	M	42	22	CMT 1 spor		12	H	
24	II.1	F	22	23	CMT 1 spor		12		
5	II.3	M	45	29	CMT 1		12		
5	II.4	F	44	42	CMT 1		11		

* Not measured in this study; classification based on previous recording.
H = homozygote.

sults remain significant for the absence of the CMT1a duplication in our sporadic CMT1a 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 I with autosomal recessive inheritance.^{26,27} 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 features of Turner's syndrome appeared and she was found to have the karyotype 46,X (iso Xq). However, she was then shown to belong to an autosomal dominant

Table 3 Charcot-Marie-Tooth type 2 cases and unaffected relatives of CMT patients, all types. NCV=NCV in the motor median nerve (m/sec). U409 was analysed in Umeå, A409 and the other probes in Antwerp. CMT2 AD = CMT type 2 autosomal dominant inheritance. CMT2 spor = sporadic cases of CMT2.

Pedigree	Age	NCV (m/sec)	A409	U409	412b	412c	401	Diagnosis
3	IV.2	34	59	23	12	12	12	CMT2 AD
3	III.1	56	49	12	12	H	12	CMT2 AD
3	III.2	54	48	H	H	H	H	CMT2 AD
3	II.4	75	52	H				CMT2 AD
8	II.1	65	48	12	H	H	H	CMT2 AD
8	III.1	41	48	H			H	CMT2 AD
17	II.2	24	45	12	H	12	12	CMT2 spor
16	II.4	31	41	12	22	12	12	CMT2 spor
23	II.2	23	48		13			CMT2 spor
2	V.23	29	54	H	12	12	H	Not affected
2	V.24	26	62	H	12	12	H	Not affected
4	II.2	60	48	H			H	Not affected
35	I.1	47	62		22			Not affected
35	I.2	45	58		12			Not affected
18	II.1	52	55		12			Not affected
18	II.2	48	57		12			Not affected
31	III.1	70	52		12			Not affected
31	III.2	65	56		12			Not affected
19	I.1	50	61		12			Not affected
19	I.2	47	55		22			Not affected
9	I.1	37	57		11			Not affected
9	I.2	37	56		11			Not affected

H = homozygote.

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 401 owing to homozygosity. 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 out 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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1 Dyck PJ. Inherited neuronal degeneration and atrophy affecting peripheral motor, sensory and autonomic neurons. In: Dyck PJ, Thomas PK, Lambert EH, Bunge R, eds. *Peripheral neuropathy*. 2nd ed. Philadelphia: Saunders, 1984:1600-42.

2 Thomas PK, Calne DB. Motor nerve conduction velocity in peroneal muscular atrophy: evidence for genetic heterogeneity. *J Neurol Neurosurg Psychiatry* 1974;37:68-75.

3 Harding AE, Thomas PK. The clinical features of hereditary motor and sensory neuropathy types I and II. *Brain* 1980;103:259-80.

4 Brust J, Lovelace R, Devi S. Clinical and electrodiagnostic feature of Charcot-Marie-Tooth syndrome. *Acta Neurol Scand Suppl* 1978;58:1-42.

5 Davis CJF, Bradley WG, Madrid R. The peroneal muscular atrophy syndrome. Clinical, genetic, electrophysiological and nerve biopsy studies. I. Clinical, genetic and electrophysiological findings and classification. *J Genet Hum* 1978;26:311-49.

6 De Visser M. Diagnostic criteria for autosomal dominant hereditary motor and sensory neuropathy type 1a. *Neuromusc Dis* 1993;3:77-9.

7 Emery AEH. Population frequencies of inherited neuromuscular diseases - a world survey. *Neuromusc Dis* 1991;1:19-29.

8 Holmberg BH. Charcot-Marie-Tooth disease in northern Sweden - an epidemiological and clinical study. *Acta Neurol Scand* 1993;87:416-22.

9 Bird TD, Ott J, Giblett ER. Evidence for linkage of Charcot-Marie-Tooth neuropathy to the Duffy locus on chromosome 1. *Am J Hum Genet* 1982;34:388-94.

10 Vance J, Barker D, Yamaoka L, et al. Localization of Charcot-Marie-Tooth disease type 1a (CMT1a) to chromosome 17p11.2. *Genomics* 1991;9:623-8.

11 Lupski J, Montes de Oca-Luna R, Slangenhuysen S, et al. DNA duplication associated with Charcot-Marie-Tooth disease type 1A. *Cell* 1991;66:219-32.

12 Raeymaekers P, Timmerman V, Nelis E, et al. Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1a (CMT1a). *Neuromusc Dis* 1991;1:93-7.

13 Raeymaekers P, Timmerman V, Nelis E, et al. Estimation of the size of the chromosome 17p11.2 duplication in Charcot-Marie-Tooth neuropathy type 1a (CMT1a). *J Med Genet* 1992;29:5-11.

14 Suter U, Welcher A, Özcelik T, et al. Trembler mouse carries a point mutation in a myelin gene. *Nature* 1992;356:241-4.

- 15 Patel P, Roa B, Welcher A, *et al.* The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot-Marie-Tooth disease type 1A. *Nature Genet* 1992;1:159-63.
- 16 Valentijn L, Bolhuis P, Zorn I, *et al.* The peripheral myelin gene PMP-22 GAS-3 is duplicated in Charcot-Marie-Tooth disease type 1A. *Nature Genet* 1992;1:166-70.
- 17 Timmerman V, Nelis E, Van Hul W, *et al.* The peripheral myelin protein gene PMP-22 is contained within the Charcot-Marie-Tooth disease type 1A duplication. *Nature Genet* 1992;1:171-5.
- 18 Matsunami N, Smith B, Ballard L, *et al.* Peripheral myelin protein-22 gene maps in the duplication in chromosome 17p11.2 associated with Charcot-Marie-Tooth 1A. *Nature Genet* 1992;1:176-9.
- 19 Dyck PJ, Karnes JL, Windebank AJ, Sparks M, Clarke-Stevens J, O'Brien PC. Minimal pathologic expression of a mutant gene for hereditary motor and sensory neuropathy. *Mayo Clin Proc* 1983;58:419-25.
- 20 Harding AE, Thomas PK. Autosomal recessive forms of hereditary motor and sensory neuropathy. *J Neurol Neurosurg Psychiatry* 1980;43:669-78.
- 21 Hoogendijk JE, Hensels GW, Gabreels-Festen AA, *et al.* De-novo mutation in hereditary motor and sensory neuropathy type I. *Lancet* 1992;339:1081-2.
- 22 Iselius L, Grimby L. A family with Charcot-Marie-Tooth disease showing a probable X-linked incompletely dominant inheritance. *Hereditas* 1982;97:157-8.
- 23 Rozear MP, Pericak-Vance MA, Fishbeck K, *et al.* Hereditary motor and sensory neuropathy, X-linked: a half century follow up. *Neurology* 1987;37:1460-5.
- 24 Ionasescu V, Trofatter J, Haines J, Summers A, Ionasescu R, Searby C. Heterogeneity in X-linked recessive Charcot-Marie-Tooth neuropathy. *Am J Hum Genet* 1991;48:1075-83.
- 25 Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*. 2nd ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 1989.
- 26 Westerberg B. *Neuropediatric aspects of hereditary peripheral neuropathy in childhood*. MD thesis, University of Gothenberg, 1982:6-36.
- 27 Ouvrier R, McLeod J, Conchin T. The hypertrophic forms of hereditary motor and sensory neuropathy. A study of hypertrophic Charcot-Marie-Tooth disease (HMSN type I) and Dejerine-Sottas disease (HMSN type III) in childhood. *Brain* 1987;100:121-48.