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Charcot–Marie–Tooth disease type 1A with 17p11.2 duplication

Clinical and electrophysiological phenotype study and factors influencing disease severity in 119 cases

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Summary

A clinical and electrophysiological study was performed in 119 Type 1A Charcot–Marie–Tooth disease (CMT1A) patients with proven 17p11.2 duplication. Onset of the first functional manifestations was in the first decade in 50% of cases and before the age of 20 years in 70% of cases. The predominant clinical signs were muscle weakness and wasting in the lower limbs. None of the patients was normal on clinical examination and all presented at least pes cavus or ankle jerk areflexia. Motor nerve conduction velocity (MNCV) was uniformly reduced in all nerves, and was $\leqslant$*33 m/s in the median nerve for all patients. Sensory potentials were abnormal in all cases, even where there was no clinical sensory loss. Needle electromyography recruitment was reduced in distal muscles for all patients. MNCV slowing was fully consistent with the presence of duplication even in clinically asymptomatic individuals or in children, confirming the complete electrophysiological penetrance of 17p11.2 duplication and making median nerve MNCV a reliable tool for screening affected at-risk individuals. Functional disability was mild. Ninety-six percent of patients were autonomous; 25% were asymptomatic and diagnosed by systematic family investigation especially on the basis of median nerve MNCV reduction. Early age at onset and greatly reduced median nerve MNCV were predictive of a more severe disease course; the earlier the onset the more reduced the median nerve MNCV and the higher the functional disability tended to be after an equivalent disease duration. Cross-sectional analysis of neurological deficit, functional deficit and MNCV according to disease duration showed that, regardless of age at onset, CMT1A disease with 17p11.2 duplication is a clinically progressive disorder. Neurological deficit and functional disability increased, whereas median nerve MNCV and compound muscle action potential (CMAP) amplitude did not change with disease course. Intrafamilial phenotype variation between parents and children and between siblings was studied in large families. Functional disability and neurological deficit differed widely and the highest range of median nerve MNCV within a family reached 23 m/s. Clinical and electrophysiological data were compared with those of CMT1B patients with peripheral myelin* P_0 *protein point mutation. CMT1A patients were found to be more severely affected with more prolonged distal motor latency and more reduced CMAP amplitude, whereas MNCV did not significantly differ, indicating that peripheral myelin* P_0 *protein point mutation is not always associated with a severe phenotype. The same genetic defect (17p11.2 duplication) results in variable expression within the phenotype, even in siblings with variations in age at onset, clinical severity and MNCV slowing. This phenotypic variation could be due to additional genetic factors related to peripheral myelin protein 22 expression as well as to other endogenous or environmental factors.*

Keywords: hereditary neuropathy; CMT1A; 17p11.2 duplication; phenotype; nerve conduction studies

Abbreviations: CMAP = compound muscle action potential; CMT = Charcot–Marie–Tooth disease; FDS = functional disability scale; GNDS = global NDS; MNCV = motor nerve conduction velocity; MNDS = motor NDS; NDS = neurological disability score; P_0 = peripheral myelin P_0 protein; PMP22 = peripheral myelin protein 22

Introduction

The syndrome of peroneal muscular atrophy was first described more than a century ago by Charcot and Marie in France (1886) and Tooth in England (1886). We are, however, indebted to Dyck and Lambert (1968) for the first comprehensive classification of the syndrome. They distinguished different types according to clinical, genetic, electrophysiological and histological characteristics. The heterogeneity of the syndrome was therefore clearly established. The absence of peroneal atrophy in some affected members of a family was observed and led Dyck to prefer the term hereditary motor and sensory neuropathy to the more restrictive term of peroneal muscular atrophy or Charcot–Marie–Tooth syndrome (CMT). A classification based on seven types was then proposed (Dyck, 1975) and was adopted by most neurologists and geneticists. Hereditary motor and sensory neuropathy Type I corresponded to the hypertrophic and more frequent form of the disease, characterized by onion bulb formation observed on nerve biopsy specimens. Hereditary motor and sensory neuropathy Type II was the neuronal form. Electrophysiological studies confirmed the discrimination between Types I and II according to the values of median motor nerve conduction velocity (MNCV) (Harding and Thomas, 1980*a*, *b*; Bouche *et al.*, 1983). The first genetic confirmation of this heterogeneity appeared in the early 1980s. Some families classified as hereditary motor and sensory neuropathy Type I were found to be linked to the Duffy locus on chromosome 1 (Bird *et al.*, 1982). Later, a duplication of a region on chromosome 17 containing the peripheral myelin protein 22 (PMP22) gene was found in numerous Type I families (Vance *et al.*, 1989; Raeymaekers *et al.*, 1989, 1991; Middleton-Price *et al.*, 1990; Lupski *et al.*, 1991; Brice *et al.*, 1992; Matsunami *et al.*, 1992; Patel *et al.*, 1992; Timmerman *et al.*, 1992). More recently, other loci have been found and a new classification has had to be proposed (Harding, 1995). The CMT eponym has now been adopted. CMT1A corresponds to patients with 17p11.2 duplication or PMP22 point-mutation, CMT1B to patients with peripheral myelin P_0 protein (P_0) mutations on chromosome 1 and CMT1C to patients where a locus has yet to be discovered (Chance *et al.*, 1990). CMT2 patients are those with the neuronal form. Other CMT types have sex-linked (CMTX) or autosomal recessive (CMT4) inheritance (Gabreëls-Festen *et al.*, 1993; Ionasescu, 1995). The CMT1A type appears to be by far the most frequent and accounts for 60–70% of CMT1 cases (Ionasescu, 1995).

CMT1A patients with the 17p11.2 duplication have the same genetic defect but present a wide range of clinical disability. A knowledge of the relationships between the phenotypic variations and other parameters, such as electrophysiological findings, age, gender, parental transmission and disease duration, may be important for understanding the physiopathology.

We report here the clinical and electrophysiological features of a large and genetically homogeneous group of CMT1 patients with 17p11.2 duplication, including a study of the disease-severity factors and intrafamilial variations within the phenotype.

Patients and methods

We studied 119 patients with proven 17p11.2 duplication. All were investigated in the same institution in Paris and examined by the same neurologist according to a defined clinical and electrophysiological protocol.

French CMT network criteria were used for diagnostic purposes; in the CMT1 type, index cases had to present (i) slowly progressive, bilateral and symmetrical distal weakness and wasting in limbs with areflexia (at least of ankle jerks) and distal sensory loss in lower extremities, and (ii) a median nerve MNCV of $\leqslant$30 m/s. At-risk relatives were considered affected if the MNCV was $\leqslant$30 m/s or $\leqslant$40 m/s on two nerves, i.e. both median nerves or the ulnar and median nerves, with all or some CMT clinical features (at least ankle jerk areflexia or pes cavus).

Clinical assessment

Age at onset was determined by questioning the patients about the age of the first symptoms such as cramps, difficulty in running, jumping, climbing stairs and walking.

A personal neurological disability score (NDS) was derived from neurological examination findings and determined as follows: (i) muscle weakness and muscle wasting, scored separately where 0 = normal, 2 = in either the upper or lower limbs, 4 = in both; (ii) upper limb tendon reflexes where 0 = normal, 1 = absent; (iii) lower limb tendon reflexes where 0 = normal, 1 = either knee jerk or ankle jerk absent, 2 = both absent; (iv) sensory loss with pain-touch and proprioception scored separately where 0 = normal, 1 = in either the upper or lower limbs, 2 = in both. The motor neurological disability score (MNDS), including muscle weakness and muscle wasting, was assessed on a scale from 0 to 8. The global neurological disability score (GNDS), including the MNDS, sensory loss and areflexia, was assessed on a scale from 0 to 15.

To determine disease severity in terms of ability to walk and run, each patient was assessed according to a nine-point functional disability scale (FDS) from 0 to 8 as follows: 0 = normal; 1 = normal, but with cramps and fatigability; 2 = inability to run; 3 = walking difficult but still possible

unaided; 4 = able to walk with a cane; 5 = able to walk with crutches; 6 = able to walk with a walker; 7 = wheelchair bound; 8 = bedridden.

In addition, all individuals were examined for the presence of foot deformities, scoliosis, nerve hypertrophy and associated signs (tremor, ataxia, pyramidal signs, deafness, optic nerve atrophy and dementia).

Electrophysiological study

Nerve conduction studies were performed with surface stimulating and recording electrodes. Distal motor latency, MNCV and distal compound muscle action potential (CMAP) were recorded from the median nerve in all patients; ulnar and peroneal nerves were studied in most of the index cases. A terminal latency index was calculated for the median nerve as follows:

$$\text{Terminal latency index} = \text{terminal distance (mm)} / [\text{MNCV (m/s)} \times \text{distal motor latency (ms)}]$$

The terminal distance was fixed at 60 mm. Terminal latency index values were compared with those of 200 normal controls.

Sensory-nerve action potentials were recorded from median and sural nerves in all index cases and, in some instances, in relatives.

Electromyography of the tibialis anterior and the first dorsalis interosseous muscles was performed with a concentric needle electrode in index patients.

The electrophysiological examination protocol was simplified for at-risk relatives if the examination was carried out at home or in children (and included at least an MNCV study on the median nerve).

Molecular study

The 17p11.2 duplication was detected by gene dosage using RFLP (restriction fragment length polymorphism) probes localized in the CMT1/hereditary neuropathy with pressure palsies monomer. The genomic probes EW 401 (D17S61) and VAW412R3 (D17S125) each detect two *Msp*I alleles 10.5 and 5.4 kb, and 5.5 and 4.4 kb long, respectively, and VAW409R3a (D17S122) detects three *Msp*I alleles of 2.8, 2.7 and 1.9 kb. Patient DNA (5 mg), digested with *Msp*I, was electrophoresed on 0.8% agarose gels, transferred to Hybond N+ membrane (Amersham, UK) and hybridized with random-primed ^{32}P-labelled probes, after preannealing with placental DNA (2 mg/l). Gene dosage was performed by visual inspection.

Statistical analysis

Percentages and means were compared using the χ^2 test and Student's *t* test, respectively; differences were considered significant for $P \leqslant 0.05$. Correlation studies were performed using simple regression analysis; the correlation was considered significant with correlation coefficient $r \geqslant 0.4$ and $P \leqslant 0.001$.

Results

Molecular diagnosis

On the basis of clinical and electrophysiological criteria, 93 CMT1 families were investigated and tested for the 17p11.2 duplication. Where no duplication was detected, P_0 mutations were sought.

Fifty-five families presented the 17p11.2 duplication and were thus classified as CMT1A; five families had P_0 point mutations. For the remaining 33 families the genetic defect could not be characterized.

Clinical and electrophysiological examinations were performed in 196 individuals belonging to the 55 CMT1A families. Of the 141 at-risk relatives, 77 were found to be affected; 64 were normal on clinical and electrophysiological examination and did not carry the duplication. The 17p11.2 duplication was detected in 132 individuals; 13 cases (belonging to five families) were excluded from the study because of incomplete clinical and/or electrophysiological data.

The remaining 119 patients with the 17p11.2 duplication therefore formed the basis of the present study.

Clinical findings

General findings

Fifty-four males and 65 females were studied. Mean age at examination was 40.2 ± 18.6 years (mean ± SD; range 2–81 years). Non-symptomatic at-risk relatives (n = 32, i.e. 27%) were systematically screened for CMT1A. Mean age at onset in symptomatic individuals was 19.4 ± 17.4 years (range 2–76 years).

The disease was transmitted from an affected father in 51 cases and from an affected mother in 43 cases. Parental transmission was unknown in the remaining cases.

Neurological examination findings

Motor and sensory loss predominated in the lower limbs in the majority of patients, foot deformities were almost invariably present and one-third of patients had kyphoscoliosis. The most frequently observed associated signs were postural hand tremor (5% of patients; n = 6) and hypoacousia (5% of patients; n = 6). No patient had pyramidal or cerebellar signs (Table 1).

GNDS findings are shown in Fig. 1A. One-third of the patients had the maximum score (GNDS = 15) with motor and sensory loss and areflexia of both the upper and lower limbs. Twenty patients (17%) had few signs (GNDS = 1–5); in most of them the neuropathy was asymtomatic and diagnosed during the familial investigation. No affected individual had

completely normal clinical findings, there was at least pes cavus and/or ankle jerk areflexia.

Functional disability was mild or absent (FDS = 0 or 1) in 41 patients (35%); 72 patients (61%) were at stage 2 or 3, with difficulty in walking or running but were still autonomous; one patient was wheelchair bound but did not differ from the patients with a high FDS (stage ⩾3) in terms of age at onset or median nerve MNCV (Fig. 1B).

Electrophysiological findings

All patients had marked slowing of motor nerve conduction (Table 2). The MNCV could not be measured in four cases (4%) for the median nerve and in 24 cases (54%) for the peroneal nerve. There was neither conduction block nor dispersion of action potentials. The CMAP amplitude was frequently reduced (87% of cases for the median nerve and 93% of cases for the peroneal and ulnar nerves). The median nerve MNCV was almost invariably ⩽30 m/s, except in four individuals (with 31, 31.5, 32 and 33 m/s) who were at-risk relatives (Fig. 1D). Median nerve terminal latency index did not differ from 183 normal controls (0.34 ± 0.1 for patients versus 0.34 ± 0.04 for controls).

Sensory action potentials were abnormal in all tested cases. They could not be recorded in 98% of cases for the sural nerve and in 85% of cases for the median nerve.

Fibrillation potentials were rarely observed (15%). Motor unit potential recruitment was reduced in all cases for the first dorsalis interosseous muscle and in 92% of cases for the tibialis anterior muscle. In 6% of cases no motor unit potential recruitment was elicited in the tibialis anterior muscle.

Correlation study

Neurological deficit, FDS and electrophysiological findings by gender and parental transmission

No significant difference was found in the GNDS, MNDS, FDS, median nerve MNCV and CMAP amplitude, between males and females, or between two groups of patients distinguished according to the gender of the affected parent.

Neurological deficit, FDS and electrophysiological findings by age at onset, age at examination, and disease duration

The median nerve MNCV was directly related to age at onset ($r = 0.47$, $P < 0.001$) (Fig. 2). Patients were subdivided into two groups according to their age at onset: an early onset group (77 patients; age at onset ⩽20 years) and a late onset group (41 patients; age ⩾21 years) (Table 3). The age of 20 years was chosen as a dividing line between the two

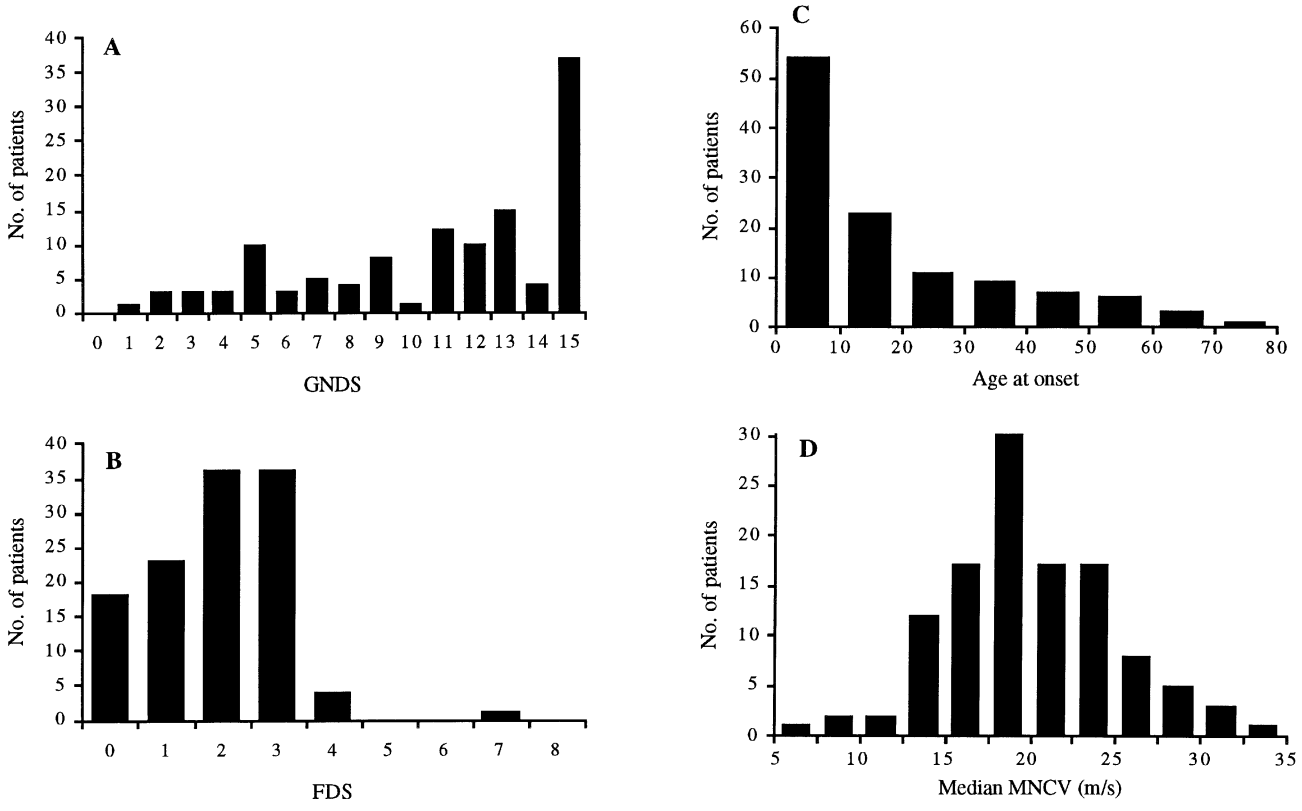

Fig. 1 Frequency distribution of (**A**) global neurological disability score (GNDS), (**B**) functional disability scale (FDS), (**C**) age at onset and (**D**) median motor nerve conduction velocity (MNCV) in 199 patients with 17p11.2 duplication.

groups for two reasons: it was close to the mean age at onset (19.4 years) and it corresponds to the upper limit of growing age. Functional disability was significantly higher in early onset patients, whose mean age at examination was significantly lower than that of the late onset group ($P < 0.01$). The disease duration was significantly longer, and the median nerve MNCV significantly more reduced, in early onset patients ($P < 0.01$). No difference was found in the GNDS and median nerve CMAP between the two groups of patients. The disease duration was compared between early and late onset patients for three different functional disability groups (Table 4). For low FDS patients (at stage 1) as well as for moderate FDS (stage 2) or high FDS (stage $\geqslant 3$) patients, disease duration was significantly longer in early onset patients. On the other hand, disease duration was significantly different between the three FDS groups, irrespective of whether they belonged to the early or late onset group. The disease appeared to have the same progression in both onset groups: FDS increased from stage 1 to stage 2 over an average evolution of 2 years, and from stage 2 to stage 3 and above over an average of 7 years. However, early onset patients remained pauci-symptomatic (FDS = 1) for a longer time than late onset patients. In addition, age at examination in each FDS group was markedly lower in early onset than in late onset patients. Early onset patients, therefore, reached high functional disability significantly earlier.

No correlation was found between either disease duration or age at examination and median nerve MNCV or CMAP amplitude (Fig. 2).

Table 1 *Clinical features in 119 CMT1 patients with 17p11.2 duplication*

Clinical features	No. of patients (%)
Muscle weakness	
Lower limbs	93 (78)
Upper limbs	73 (61)
Muscle wasting	
Lower limbs	105 (88)
Upper limbs	84 (71)
Sensory loss	
Pain and touch	76 (64)
Proprioception	82 (69)
Areflexia	
Lower limbs	115 (97)
Upper limbs	94 (79)
Foot deformities	113 (95)
Scoliosis	42 (35)

Table 2 *Motor nerve conduction findings in 119 CMT1A patients with 17p11.2 duplication*

	Mean ± SD	Range	Unrecordable
Median nerve ($n = 119$)			4
DML (ms)	9.8 ± 2.6	4.6–22.3	
CMAP (mV)	2.4 ± 1.9	0.1–9.5	
MNCV (m/s)	20.2 ± 4.9	7–33	
TLI	0.34 ± 0.1	0.17–0.68	
Ulnar nerve ($n = 50$)			0
DML (ms)	8 ± 3.1	4.5–16.4	
CMAP (mV)	2.4 ± 1.6	0.04–8.5	
MNCV (m/s)	17.3 ± 5.1	6–30	
Peroneal nerve ($n = 54$)			24
DML (ms)	11.9 ± 3.4	6–19.4	
CMAP (mV)	0.9 ± 1.2	0.07–4	
MNCV (m/s)	17 ± 4.6	5–24	

DML = distal motor latency; CMAP = compound muscle action potential; MNCV = motor nerve conduction velocity; TLI = terminal latency index.

FDS, clinical and electrophysiological findings

Clinical and electrophysiological data were compared between three FDS groups: low (stage 0 or 1), moderate (stage 2) and high (stage $\geqslant 3$) (Table 5). Unpaired *t* test of MNCV between the low FDS group on the one hand and the moderate and high FDS groups on the other showed a significant difference: it was less reduced in low FDS patients ($P < 0.05$). No difference was found in the median nerve MNCV between moderate and high FDS groups or in the median nerve CMAP between the three FDS groups. Both low and moderate FDS patients were significantly younger than those with a high FDS. Onset was significantly later in low FDS than in moderate FDS patients. Both moderate and high FDS patients had early onset but functional disability increased with disease duration, which was more prolonged in high FDS patients. Moderate and high FDS patients differed only in terms of disease duration. Symptomatic patients could therefore be defined by an FDS $\geqslant 2$; they were markedly different from a- or pauci-symptomatic patients (FDS = 0 or 1) in age at onset, disease duration, neurological disability and median nerve MNCV.

Clinical and electrophysiological variations within the same family

Eight families with four to eight affected members were studied (Fig. 3). Median nerve MNCV varied considerably within families (lowest value 9.5 m/s, highest value 33 m/s). The mean MNCV for the eight families was 20.8 ± 5.9 m/s. The mean difference between the highest and lowest median nerve MNCV values in each family was 13.1 m/s (range 4.5–23 m/s). The difference in conduction velocity between parent and child was >15 m/s in one instance and >10 m/s in four instances out of 26 pairs. Within siblings the difference in conduction velocity was >15 m/s in two instances and >10 m/s in seven instances out of 28 pairs. The GNDS and FDS were also variable; the GNDS ranged from 5 to 14 and the FDS from 1 to 4 in the eight families.

Comparison with patients with P_0 mutations

All clinical and electrophysiological data were compared with those of 10 patients belonging to five families with

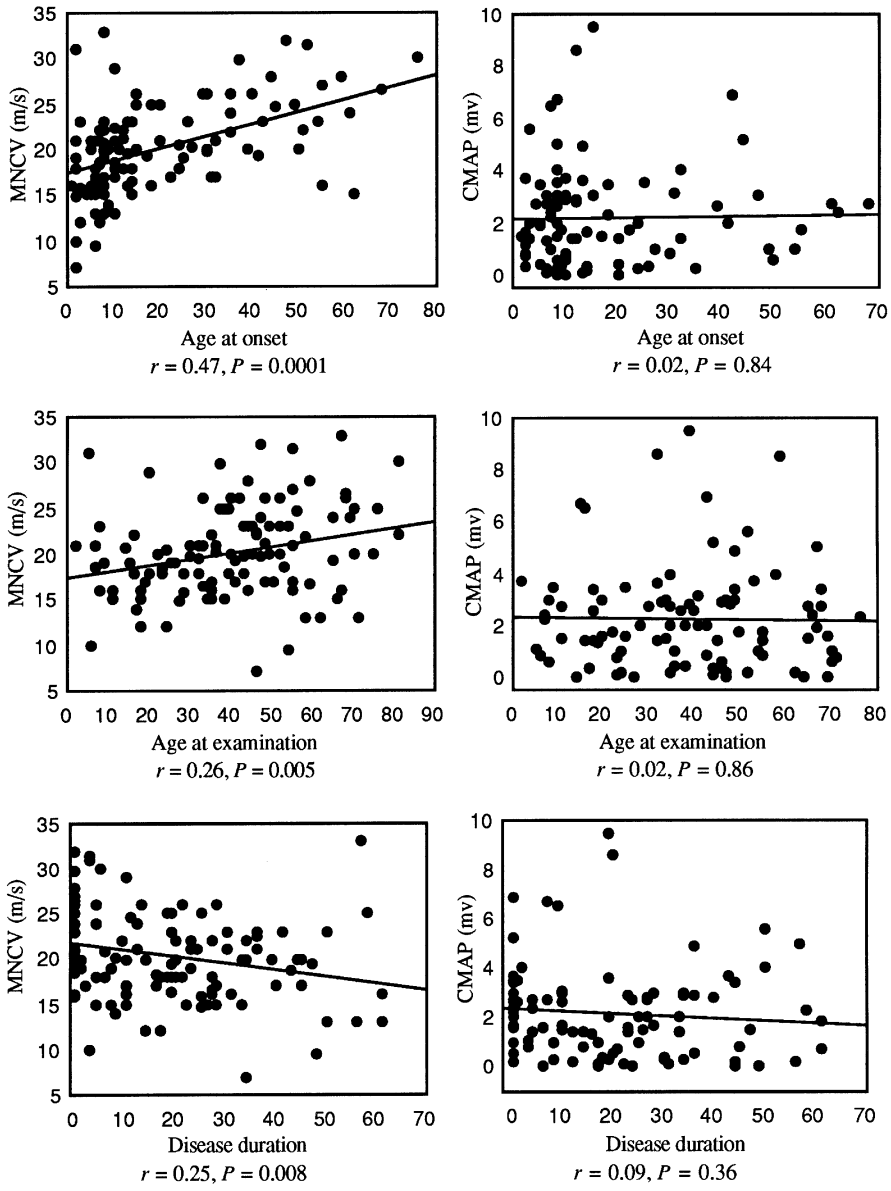

Fig. 2 Scatter diagrams with regression analysis between age at onset, age at examination and disease duration on the one hand and median nerve MNCV (in m/s) and median nerve CMAP (in mV) on the other.

P_0 point mutation reported previously by Rouger *et al.* (1996). The GNDS and MNDS were significantly higher in CMT1A patients. No differences were found in FDS or MNCV of median ulnar and peroneal nerves. However, distal motor latencies were significantly greater and CMAP amplitudes significantly lower in CMT1A patients. No differences were found in sensory action potentials or electromyography recruitment (Table 6).

Discussion

The present group of CMT1A patients with 17 p11.2 duplication is the largest to be reported so far. This genetically homogeneous group underwent a detailed clinical and electrophysiological analysis and a study of the relationship between the severity of the disorder on the one hand and clinical and electrophysiological data on the other.

Clinical and electrophysiological characteristics

In our patients with CMT1A and in previous large series of CMT1 cases (Dyck 1975; Buchthal and Behse, 1977; Harding and Thomas, 1980*a*), onset was in the first decade in 50% of cases and before the age of 20 years in 70% of patients (Fig. 1C). The age at onset was determined by questioning the patients about their first disabling symptoms; hence, it characterized the first functional manifestations rather than the onset of the disorder process itself, which could have begun in early childhood or even before. The fact that CMT1 is a slowly progressive disorder could make the time of onset

Table 3 *Clinical and electrophysiological comparison between two age-at-onset groups of CMT1A patients with 17p11.2 duplication*

Parameter	Age-at-onset group		Probability
	⩽20 years (Group I)	⩽20 years (Group II)	
No. of patients	77	37	
Age at examination (years)	34.5 ± 18.2	50.4 ± 15	$P < 0.01$
Disease duration (years)	27 ± 15.6	14.5 ± 10.6	$P < 0.01$
GNDS	11 ± 4	10 ± 4	n.s.
FDS			$P < 0.01$
Low (0 or 1)	22.7%	58.5%	
Moderate (2)	36%	22%	
High (⩾3)	41.3%	19.5%	
Median MNCV (m/s)	18.5 ± 4.6	22.9 ± 4.3	$P < 0.01$
Median CMAP (mV)	2.2 ± 1.9	2.2 ± 1.5	n.s.

Data are shown as percentages or as means ± SDs. GNDS = global neurological disability score; FDS = functional disability score; n.s.= not significant.

Table 4 *Comparison of disease duration and age at examination between early and late onset patients for three different functional disability groups*

Parameter	Early disease onset (⩽20 years)	Late disease onset (⩽20 years)	Probability
Disease duration			
Mild FDS (1)	22.4 ± 18.3 ($n = 11$)	8.8 ± 9.4 ($n = 12$)	$P < 0.05$
Moderate FDS (2)	24.4 ± 13.7 ($n = 27$)	10.7 ± 9.2 ($n = 8$)	$P < 0.05$
High FDS (⩾3)	30.9 ± 16.5 ($n = 31$)	17.7 ± 14 ($n = 8$)	$P < 0.05$
Age at examination			
Mild FDS (1)	27.8 ± 19 ($n = 11$)	49 ± 8.6 ($n = 12$)	$P < 0.01$
Moderate FDS (2)	33.3 ± 14.6 ($n = 27$)	50 ± 13.7 ($n = 8$)	$P < 0.01$
High FDS (⩾3)	42 ± 18 ($n = 31$)	67 ± 12 ($n = 8$)	$P < 0.001$

Data are presented as means ± SDs.

Table 5 *Clinical and electrophysiological comparisons between three FDS groups of CMT1A patients with 17p11.2 duplication*

Parameter	FDS score			I versus II	I versus III	II versus III
	Low (0 or 1) (Group I)	Moderate (2) Group II	High (⩾3) Group III			
No. of patients	41	36	40			
Age at onset (years)	24.6 ± 18.7	15.5 ± 15.2	18.6 ± 17.5	$P < 0.05$	n.s.	n.s.
Age at examination (years)	35.9 ± 18.8	36.8 ± 15.8	47.4 ± 19.6	n.s.	$P < 0.01$	$P < 0.01$
Disease duration (years)	9.2 ± 14.1	21.2 ± 14	28.2 ± 16.7	$P < 0.01$	$P < 0.01$	$P = 0.05$
GNDS	8 ± 4	12 ± 3	12 ± 3	$P < 0.01$	$P < 0.01$	n.s.
Median MNCV (m/s)	22.1 ± 4.7	18.5 ± 4.8	19.5 ± 4.6	$P < 0.01$	$P < 0.05$	n.s.
Median CMAP (mV)	2.6 ± 1.7	1.9 ± 1.5	2.1 ± 2.5	n.s.	n.s.	n.s.

Mean ± SD, n.s.= not significant.

imprecise, especially in older patients who may not remember precisely the age at which the first symptoms occurred. In asymptomatic patients, the age at onset cannot be determined. Such problems in timing the onset were emphasized by Harding and Thomas (1980*a*). In our study, detailed questions about the patient's age at the time of the first symptoms, such as cramps, difficulty in running, jumping and climbing stairs, allowed us to date as precisely as possible the onset of the disorder in 114 patients. The close correlation in distribution of age at onset between the reported groups of CMT1 patients would seem to confirm the validity of the method used to determine this parameter.

Clinical features were dominated by areflexia and foot deformities (omnipresent), and by muscle weakness and wasting, which predominated in the lower limbs. Sensory loss, dominated by proprioception loss, was noted in >75%

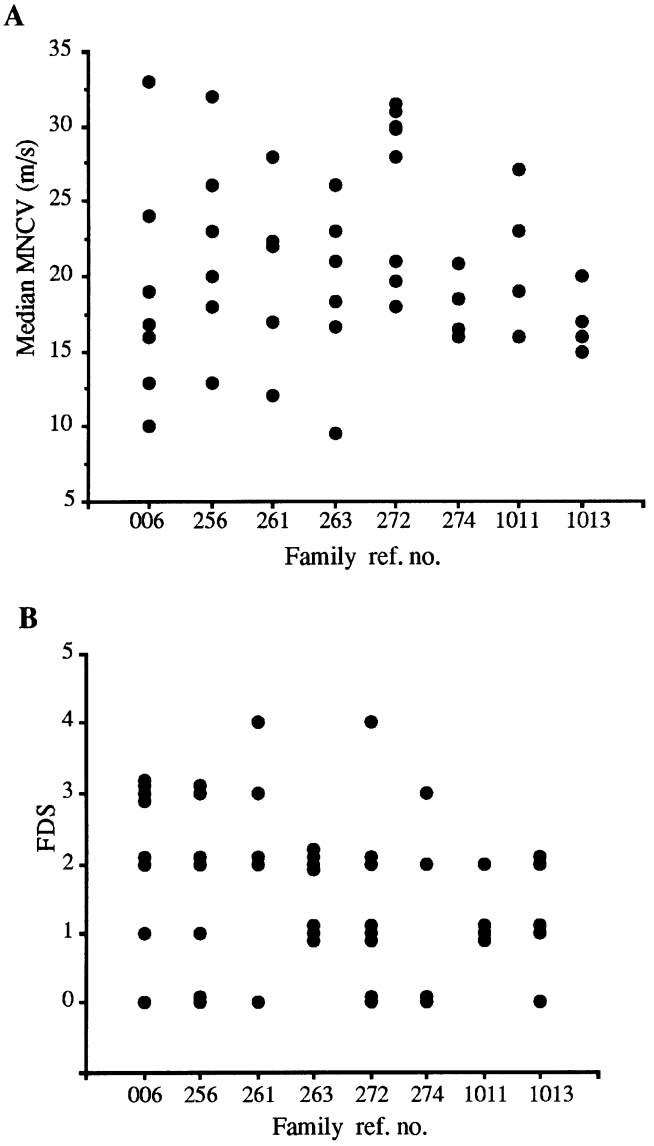

Fig. 3 (**A**) Median motor nerve conduction velocity (MNCV) and (**B**) functional disability scale (FDS) in members of eight large families with CMT1A disase with 17p11.2 duplication, studied for intrafamilial variations.

of patients. Associated signs were rare. Neither cerebellar nor pyramidal signs were observed. These data did not differ from other large groups of CMT1 patients (Dyck and Lambert, 1968; Harding and Thomas, 1980*a*; Berciano *et al.*, 1989) or the smaller series of CMT1A patients with 17p11.2 duplication (Hoogendijk *et al.*, 1994). Proximal motor deficit has rarely been reported and was not observed in our patients, although Hoogendijk *et al.* (1994) reported proximal paresis in 11% of cases.

Functional disability was judged on a simple scale based on the ability to walk and run. Severe disability was rarely observed. Only four individuals (3.4%) needed a cane for walking, and one patient was wheelchair bound. Patients became truly symptomatic with the onset of running difficulties (FDS = 2), two different groups of patients could

Table 6 *Clinical and electrophysiological comparison between CMT1A patients with 17p11.2 duplication and CMT1B patients with P_0 point mutation*

	CMT1A	CMT1B	Probability
No. of patients	119	10	
Age at onset (years)	19.4 ± 17.4	13.6 ± 8.7	n.s.
Disease duration (years)	19.7 ± 16.9	9.6 ± 11.7	n.s.
MNDS	6 ± 2	4 ± 2	$P < 0.05$
GNDS	11 ± 4	7 ± 3	$P < 0.01$
FDS			n.s.
Low (0 or 1)	35%	60%	
Moderate (2)	30.7%	10%	
High (⩾3)	34.3%	30%	
Median DML (ms)	9.8 ± 2.6	5.3 ± 1.7	$P < 0.001$
Median CMAP (mV)	2.2 ± 1.9	5.4 ± 2.7	$P < 0.001$
Median MNCV (m/s)	20.2 ± 4.9	19.7 ± 6.8	n.s.

Mean ± SD; n.s.= not significant.

then be distinguished according to the severity of functional disability: asymptomatic patients (FDS = 0 or 1) and symptomatic patients (FDS ⩾ 2). Symptomatic patients had earlier onset, longer disease duration, a higher NDS and an even slower nerve conduction than asymptomatic patients. Our FDS was reliable for determining disease severity in the lower limbs whether it was related to motor deficit or to ataxia due to proprioception loss, which could be severe in some instances.

As reported previously (Berciano *et al.*, 1989; Nicholson, 1991; Kaku *et al.*, 1993*a*), MNCV constitutes a reliable tool for screening affected at-risk relatives. Conduction slowing was fully consistent with DNA analysis, even in children and clinically asymptomatic individuals. Eight children were diagnosed before the age of 10 years (range 2–9 years); all of them had an evident CMT1 phenotype with a reduced median nerve MNCV ranging from 10 to 31 m/s. The youngest was a 2-year-old girl with muscle wasting and weakness of the lower limbs, ankle jerk areflexia and a median nerve MNCV of 21 m/s. The CMT1A diagnosis was obtained by systematic examination of 32 out of 77 affected at-risk relatives, especially on the basis of slower MNCVs. These results confirm the complete electrophysiological penetrance of CMT1A with 17p11.2 duplication.

The highest value for the median nerve MNCV was 33 m/s, whereas in other series of CMT1A patients with 17p11.2 duplication it reached 38 or 42 m/s (Nicholson, 1991; Kaku *et al.*, 1993*a*). This difference could be due to our electrophysiological criteria, but 35 families with intermediate MNCV (30 to 40 m/s) in the index patient did not carry the 17p11.2 duplication.

The median nerve terminal latency index, which explores the ratio between distal and proximal conduction velocity (Kaku *et al.*, 1994), did not differ from that in controls. In addition, neither conduction block nor temporal dispersion of action potentials was observed. These data confirm the uniform slowing of motor nerve conduction between proximal and distal segments. Sensory nerve potentials were abnormal

in all patients, and were abolished in most cases even though patients did not have clinical sensory loss. All previous electrophysiological descriptions of CMT1 have underlined these characteristics (Buchthal and Behse, 1977; Harding and Thomas, 1980*a*; Bouche *et al.*, 1983; Ouvrier *et al.*, 1987; Kaku *et al.*, 1993*a*, *b*).

Clinical and electrophysiological characteristics of our CMT1A patients did not significantly differ from those previously reported in CMT1 patients without molecular diagnosis or CMT1A patients with proven 17p11.2 duplication. This could be due to the fact that the duplication in 17p11.2 is the most frequent molecular defect in CMT1. In contrast with the results of Dyck *et al.* (1989), we found that the disease is more severe in CMT1A patients than in CMT1B patients. This divergence may be explained by the smaller number of CMT1B patients in our study and the fact that CMT1A patients in the study of Dyck *et al.* (1989) corresponded to patients without linkage to the Duffy locus, and so could include CMT1A and CMT1C patients in the most recent classification. However, our results demonstrate that CMT1B is not always associated with a severe phenotype.

Correlation study and disease-severity factors

Previous reports underlined phenotype variations in CMT1 patients and tried to establish a causal relation between electrophysiological characteristics and clinical severity (Dyck *et al.*, 1989; Nicholson, 1991; Kaku *et al.*, 1993*a*; Hoogendijk *et al.*, 1994; Killian *et al.*, 1996). Few studies have tried to find an association between age at onset and severity of the disorder. Harding and Thomas (1980*a*) considered that age of onset was not a reliable indicator of disease severity. Our study suggests that functional disability is related to disease duration. However, disease course is dependent on age at onset. Indeed, patients with onset before the age of 20 years remained pauci-symptomatic much longer than late onset patients. This could be due to factors relating to remyelination/regeneration capabilities in younger patients. Indeed, serial biopsy studies reported an increase in the number and size of onion bulb formations with age (Meier *et al.*, 1976; Ouvrier *et al.*, 1987; Gabreëls-Festen *et al.*, 1992), but they did not give a description of axonal status. In the late stages (FDS $\geqslant$ 2), the progression is similar in early and late onset groups. However, early onset patients can reach a high FDS stage relatively early, compared with late onset patients, since the latter developed the disease after the fourth decade in 46% of cases and the former during the first decade in 70% of cases. The factors that could explain such differences in onset, which exist even among siblings, have yet to be determined; in this respect, additional hypothetical genetic mechanisms related to PMP22 expression could prevent the early manifestation of the disorder in some individuals.

The absence of any correlation between the MNCV and clinical severity in CMT1 patients was underlined by previous investigators (Dyck and Lambert, 1968; Harding and Thomas, 1980*a*; Bouche *et al.*, 1983). In our study of CMT1A patients, the median nerve MNCV was significantly more reduced in patients with high functional disability. These data appear to confirm those of Hoogendijk *et al.* (1994), who found that the MNCV was inversely related to neurological disability, though there were differences in the assessment scale used in the two studies; in our study functional disability was determined by the effect of the disorder on the ability to walk and run, whereas in previous reports, clinical severity was evaluated using a neurological disability scale derived from a neurological examination especially designed for this purpose. Our cross-sectional analysis findings and those of Hoogendijk *et al.* (1994) are close to those in a longitudinal study reported by Dyck *et al.* (1989) of 31 patients (one CMT1B patient with linkage to the Duffy locus; and 30 CMT1 patients without linkage to the Duffy locus), which showed that the severity of conduction-velocity abnormality predicted clinical severity, since it was significantly associated with the NDS and subsets of the NDS. They also found that the CMAP amplitude tended to decrease with age more frequently in the peroneal nerve than in the ulnar and median nerves. We were unable to perform the same cross-sectional analysis with peroneal MNCVs and CMAPs because no response could be elicited in peroneal nerve in 50% of cases, making the number of patients in each subgroup (determined by age at onset or FDS) too small for reliable statistical analysis. Killian *et al.* (1996) reported a longitudinal evaluation of nerve conduction within a single family with 17p11.2 duplication over a period of 22 years. The MNCV did not change significantly whereas functional worsening was noticed in half of the patients; age at onset was not, however, specified for these patients. The clinical progression of the disorder could be due to other, unknown factors, probably related to the demyelination/remyelination process rather than to axonal loss, as previously stated by Roy *et al.* (1989) in a longitudinal study performed in 10 CMT1 patients which found a decrease in the median nerve CMAP with no significant change in the MNCV or distal motor latency. We found no difference in median nerve CMAP amplitudes between the three FDS groups and no correlation was found between the median nerve CMAP and disease duration or age at examination. As CMT1A is a slowly progressive disorder, a possible regeneration process could explain the absence of a decrease in CMAP amplitude during the course of the disease.

Regardless of age at onset, CMT1A with 17p11.2 duplication is a progressive disorder, but early onset is highly correlated with a high functional disability and low MNCV. Age at onset and median nerve MNCV combined could, therefore, be valuable prognostic factors in CMT1A patients.

Intrafamilial variation within the CMT1A phenotype

The phenotypical expression of the same genetic defect is extremely variable. Within the same family, clinical severity

and median nerve MNCV can differ, with a wide range between siblings as well as between parents and children, as reported by Kaku *et al.* (1993*a*). The pathogenesis of this variation remains unclear. Furthermore, Garcia *et al.* (1995) reported clinical variation in two pairs of identical twins. Phenotypical variability could be due to other genetic factors, such as modulator genes, as well as to endogeneous or environmental factors.

Conclusions

We conclude that MNCV slowing is a reliable tool in identifiying affected individuals; median nerve MNCV is invariably ⩽33 m/s. Nerve conduction velocity is uniformly reduced in all nerves and equally along nerve segments. CMT1A severity is frequently mild or moderate and the phenotype is variable even between siblings, these findings are of particular relevance to genetic counselling. Age at onset is a reliable tool to determine disease severity; the earlier the onset, the lower the MNCV and the higher the functional disability. Although CMT1A is a clinically progressive disorder, the MNCV and CMAP do not change significantly during the course of the disease. The main myelin and axon damage, responsible for nerve conduction slowing, may occur during early childhood. A better knowledge of the regulation of PMP22 expression, of its function in Schwann cells and in Schwann cell/axon interaction may help us to understand the mechanisms leading to late onset of the disorder in some affected individuals, the characteristics of disease progression and the phenotype variability of 17p11.2 duplication.

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References

Berciano J, Combarros O, Calleja J, Polo JM, Leno C. The application of nerve conduction and clinical studies to genetic counseling in hereditary motor and sensory neuropathy type I. Muscle Nerve 1989; 12: 302–6.

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.

Bouche P, Gherardi R, Cathala HP, Lhermitte F, Castaigne P. Peroneal muscular atrophy. Part I. Clinical and electrophysiological study. J Neurol Sci 1983; 61: 389–99.

Brice A, Ravisé N, Stevanin G, Gugenheim M, Bouche P, Penet C, et al. Duplication within chromosome 17p11.2 in 12 families of French ancestry with Charcot–Marie–Tooth disease type Ia. J Med Genet 1992; 29: 807–12.

Buchthal F, Behse F. Peroneal muscular atrophy (PMA) and related disorders. I. Clinical manifestations as related to biopsy findings, nerve conduction and electromyography. Brain 1977; 100: 41–66.

Chance PF, Bird TD, O'Connell P, Lipe H, Lalouel JM, Leppert M. Genetic linkage and heterogeneity in type I Charcot–Marie–Tooth disease (hereditary motor and sensory neuropathy type I). Am J Hum Genet 1990; 47: 915–25.

Charcot JM, Marie P. Sur une forme particulière d'atrophie musculaire progressive, souvent familiale, débutant par les pieds et les jambes et atteignant plus tard les mains. Rev Méd Paris 1886; 6: 97–138.

Dyck PJ. Inherited neuronal degeneration and atrophy affecting peripheral motor, sensory, and autonomic neurons. In: Dyck PJ, Thomas PK, Lambert EH, editors. Peripheral neuropathy. Philadelphia: W. B. Saunders, 1975: 825–67.

Dyck PJ, Lambert EH. Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. I. Neurologic, genetic, and electrophysiologic findings in hereditary polyneuropathies. Arch Neurol 1968; 18: 603–18.

Dyck PJ, Karnes JL, Lambert EH. Longitudinal study of neuropathic deficits and nerve conduction abnormalities in hereditary motor and sensory neuropathy type I. Neurology 1989; 39: 1302–8.

Gabreëls-Festen AAWM, Joosten EMG, Gabreëls FJM, Jennekens FGI, Janssen-van Kempen TW. Early morphological features in dominantly inherited demyelinating motor and sensory neuropathy (HMSN type I). J Neurol Sci 1992; 107: 145–54.

Gabreëls-Festen AA, Gabreëls FJ, Jennekens FG. Hereditary motor and sensory neuropathies, present status of types I, II and III. [Review]. Clin Neurol Neurosurg 1993; 95: 93–107.

Garcia CA, Malamut RE, England JD, Parry GS, Liu P, Lupski JR. Clinical variability in two pairs of identical twins with the Charcot–Marie–Tooth disease type 1A duplication. Neurology 1995; 45: 2090–3.

Harding AE. From the syndrome of Charcot, Marie and Tooth to disorders of peripheral myelin proteins. [Review]. Brain 1995; 118: 809–18.

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

Harding AE, Thomas PK. Genetic aspects of hereditary motor and sensory neuropathy (types I and II). J Med Genet 1980b; 17: 329–36.

Hoogendijk JE, De Visser M, Bolhuis PA, Hart AA, Ongerboer de Visser BW. Hereditary motor and sensory neuropathy type I: clinical and neurographical features of the 17p duplication subtype. Muscle Nerve 1994; 17: 85–90.

Ionasescu VV. Charcot–Marie–Tooth neuropathies: from clinical description to molecular genetics [see comments]. [Review]. Muscle Nerve 1995; 18: 267–75. Comment in: Muscle Nerve 1995; 18: 1215–16.

Kaku DA, Parry GJ, Malamut R, Lupski JR, Garcia CA. Nerve

conduction studies in Charcot–Marie–Tooth polyneuropathy associated with a segmental duplication of chromosome 17 [see comments]. Neurology 1993a; 43: 1806–8. Comment in: Neurology 1994; 44: 1985–6.

Kaku DA, Parry GJ, Malamut R, Lupski JR, Garcia CA. Uniform slowing of conduction velocities in Charcot–Marie–Tooth polyneuropathy type 1. Neurology 1993b; 43: 2664–7.

Kaku DA, England JD, Sumner AJ. Distal accentuation of conduction slowing in polyneuropathy associated with antibodies to myelin-associated glycoprotein and sulphated glucuronyl paragloboside. Brain 1994; 117: 941–7.

Killian JM, Tiwari PS, Jacobson S, Jackson RD, Lupski JR. Longitudinal studies of the duplication form of Charcot–Marie–Tooth polyneuropathy. Muscle Nerve 1996; 19: 74–8.

Lupski JR, de Oca-Luna RM, Slaugenhaupt S, Pentao L, Guzzetta V, Trask BJ, et al. DNA duplication associated with Charcot–Marie–Tooth disease type IA. Cell 1991; 66: 219–32.

Matsunami N, Smith B, Ballard L, Lensch MW, Robertson M, Albertsen H, et al. Peripheral myelin protein-22 gene maps in the duplication in chromosome 17p11.2 associated with Charcot–Marie–Tooth 1A. Nat Genet 1992; 1: 176–9.

Meier C, Maibach R, Isler W, Bischoff A. Dynamic aspects of peripheral nerve changes in progressive neural muscular atrophy: light- and electronmicroscopic studies of serial nerve biopsies. J Neurol 1976; 211: 111–24.

Middleton-Price HR, Harding AE, Monteiro C, Berciano J, Malcolm S. Linkage of hereditary motor and sensory neuropathy type I to the pericentromeric region of chromosome 17. Am J Hum Genet 1990; 46: 92–4.

Nicholson GA. Penetrance of the hereditary motor and sensory neuropathy Ia mutation: assessment by nerve conduction studies. Neurology 1991; 41: 547–52.

Ouvrier RA, McLeod JG, Conchin TE. 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; 110: 121–48.

Patel PI, Roa BB, Welcher AA, Schoener-Scott R, Trask BJ, Pentao L, et al. The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot–Marie–Tooth disease type IA. Nat Genet 1992; 1: 159–65.

Raeymaekers P, Timmerman V, De Jonghe P, Swerts L, Gheuens J, Martin JJ, et al. Localization of the mutation in an extended family with Charcot–Marie–Tooth neuropathy (HMSN I). Am J Hum Genet 1989; 45: 953–8.

Raeymaekers P, Timmerman V, Nelis E, De Jonghe P, Hoogendijk JE, Baas F, et al. Duplication in chromosome 17p11.2 in Charcot–Marie–Tooth neuropathy type Ia (CMT 1a). Neuromuscul Disord 1991; 1: 93–7.

Rouger H, Le Guern E, Gouider R, Tardieu S, Birouk N, Gugenheim M, et al. High frequency of mutations in codon 98 of the peripheral myelin protein P0 gene in 20 French CMT1 patients [letter]. Am J Hum Genet 1996; 58: 638–41.

Roy EP 3d, Gutmann L, Riggs JE. Longitudinal conduction studies in hereditary motor and sensory neuropathy type 1. Muscle Nerve 1989; 12: 52–5.

Timmerman V, Nelis E, Van Hul W, Nieuwenhuijsen BW, Chen KL, Wang S, et al. The peripheral myelin protein gene PMP-22 is contained within the Charcot–Marie–Tooth disease type IA duplication [published erratum appears in Nat Genet 1992; 2: 84]. Nat Genet 1992; 1: 171–5.

Tooth HH. The peroneal type of progressive muscular atrophy. London: H.K. Lewis, 1886.

Vance JM, Nicholson GA, Yamaoka LH, Stajich J, Stewart CS, Speer MC, et al. Linkage of Charcot–Marie–Tooth neuropathy type Ia to chromosome 17. Exp Neurol 1989; 104: 186–9.

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