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Neurological dysfunction and axonal degeneration in Charcot–Marie–Tooth disease type 1A

Karen M. Krajewski,[1,2] Richard A. Lewis,[1] Darren R. Fuerst,[3] Cheryl Turansky,[1] Steven R. Hinderer,[4] James Garbern,[1,2] John Kamholz[1,2] and Michael E. Shy[1,2]

[1]*Department of Neurology,* [2]*Center for Molecular Medicine and Genetics and* [3]*Department of Psychiatry and Behavioral Sciences, Wayne State University School of Medicine and* [4]*Rehabilitation Institute of Michigan, Wayne State University, Detroit, Michigan, USA*

Correspondence to: Michael E. Shy, MD, Department of Neurology, Wayne State University School of Medicine, Elliman Building, Room 3301, 421 E. Canfield, Detroit, MI 48201, USA
E-mail: m_shy@mail4.wayne.edu

Summary

Charcot–Marie–Tooth disease type 1A (CMT1A), the most frequent form of CMT, is caused by a 1.5 Mb duplication on the short arm of chromosome 17. Patients with CMT1A typically have slowed nerve conduction velocities (NCVs), reduced compound motor and sensory nerve action potentials (CMAPs and SNAPs), distal weakness, sensory loss and decreased reflexes. In order to understand further the molecular pathogenesis of CMT1A, as well as to determine which features correlate with neurological dysfunction and might thus be amenable to treatment, we evaluated the clinical and electrophysiological phenotype in 42 patients with CMT1A. In these patients, muscle weakness, CMAP amplitudes and motor unit number estimates correlated with clinical disability, while motor NCV did not. In addition, loss of joint position sense and reduction in SNAP amplitudes also correlated with clinical disability, while sensory NCV did not. Taken together, these data strongly support the hypothesis that neurological dysfunction and clinical disability in CMT1A are caused by loss or damage to large calibre motor and sensory axons. Therapeutic approaches to ameliorate disability in CMT1A, as in amyotrophic lateral sclerosis and other neurodegenerative diseases, should thus be directed towards preventing axonal degeneration and/or promoting axonal regeneration.

Keywords: CMT; demyelination; axonal degeneration; nerve conduction velocities

Abbreviations: AI = ambulation index; APB = abductor pollicus brevis; CMAP = compound motor action potential; CMT = Charcot–Marie–Tooth disease; MNCV = motor nerve conduction velocity; MUNE = motor unit number estimate; NCV = nerve conduction velocity; QMT = quantitative motor testing; QST = quantitative sensory testing; SNAP = sensory nerve action potential; SNCV = sensory nerve conduction velocity

Introduction

Charcot–Marie–Tooth disease (CMT) is among the most common inherited neurological disorders, with a prevalence reported as high as 36 per 100 000 (Skre, 1974). The syndrome was initially described by Charcot and Marie in France (Charcot and Marie, 1886) and Tooth in England (Tooth, 1886) and confirmed to be a disorder of the PNS by Hoffmann (Hoffmann, 1889). All these early investigators appreciated the fact that the disease was hereditary and often had its onset in childhood. In addition, they also recognized the characteristic clinical phenotype of distal leg weakness and atrophy, decreased sensation and absent reflexes. Little new was added to this description until the late 1950s, when Gilliatt and Thomas noted that some patients with CMT had slow nerve conduction velocities (NCVs) (Gilliatt and Thomas, 1957). Later, in the 1960s and 1970s, several groups also found that CMT patients could be divided into two classes: those with slow and those with normal NCV (CMT1 and CMT2, respectively) (Dyck and Lambert, 1968; Dyck *et al*., 1968; Thomas and Calne, 1974; Buchthal and Behse, 1977; Brust *et al*., 1978). In an evaluation of over 200 patients with CMT, Harding and Thomas demonstrated further that CMT1 and CMT2 both segregated mainly as an autosomal dominant trait (Harding and Thomas, 1980*b*), and confirmed the clinical description of the disease by Charcot, Marie and Tooth (Harding and Thomas, 1980*a*). Finally, Lewis and Sumner showed that CMT1 patients have uniformly slowed NCVs, in contrast with patients with acquired demyelinating neuropathies such as the Guillain–Barré syndrome or CIDP (chronic inflammatory demyelinating polyneuropathy) (Lewis and Sumner, 1982).

Taken together, these studies provide a relatively complete clinical description of CMT which can be used to distinguish it from other neuropathies.

Recent investigations into the genetic aetiology of the demyelinating form of CMT, CMT1, have demonstrated that the cause of the disease is heterogeneous. Bird and colleagues performed one of the first human genetic linkage studies on several families with CMT1 and showed that their disease was linked to the Duffy blood group locus on chromosome 1 (Bird *et al*., 1982). Most families with CMT1, however, are not linked to the chromosome 1 locus, but rather to a region on the short arm of chromosome 17 (Raeymaekers *et al*., 1989; Vance *et al*., 1989; Middleton-Price *et al*., 1990). In 1991, two groups showed that this form of CMT1, now called CMT1A, was associated with a 1.5 Mb duplication on the short arm of chromosome 17 (Lupski *et al*., 1991; Raeymaekers *et al*., 1991), which is now known to account for up to 80% of patients with CMT (Wise *et al*., 1993; Nelis *et al*., 1996). CMT1 linked to chromosome 1, now called CMT1B, is less common (Nelis *et al*., 1996) and is caused by mutations in the gene encoding the major PNS myelin structural protein, P0 (Hayasaka *et al*., 1993). Duplication of the PMP22 (peripheral myelin protein 22) gene, or CMT1A, thus accounts for most cases of demyelinating CMT and we will focus the rest of this paper on this entity.

Although the clinical and electrophysiological manifestations of CMT1A have been well described and its major genetic cause delineated, much remains unknown. The natural history of the disease, for example, has not been described in detail. The onset and rate of progression of weakness and sensory loss are not known and may be quite variable both between and among families (Thomas *et al*., 1997), including affected identical twins (Kaku *et al*., 1993*b*). Obtaining accurate natural history data for patients with CMT1A is important, both for understanding the molecular pathogenesis of the disease and for future developments of molecular therapies.

The cellular and molecular mechanisms underlying the onset of weakness in patients with CMT1A are also not known. NCVs, a marker of demyelination in CMT (Behse and Buchthal, 1977; Buchthal and Behse, 1977), are maximally slowed by 5 years of age in patients with CMT1A (Nicholson, 1991; Garcia *et al*., 1998), preceding the onset of weakness and disability, suggesting that demyelination *per se* is not the cause. In fact, in several studies, disability in CMT1A appeared to correlate better with axonal degeneration, since weak muscles were typically atrophied and pathological analysis of nerve biopsies of patients demonstrated axonal degeneration (Davis *et al*., 1968, 1978; Bouche *et al*., 1983; Dyck, 1984; Combarros *et al*., 1987; Berciano *et al*., 1989). Consistent with this hypothesis, Sahenk and colleagues have shown that xenograft transplants of CMT1A Schwann cells into sciatic nerve of nude mice reduced the calibre of regenerating axons (Sahenk *et al*., 1999). A recent study of several Dutch families with CMT1A (Hoogendijk *et al*., 1994), however, demonstrated that weakness correlated best with median motor NCV (MNCV), a marker of demyelination, rather than with median compound motor action potential (CMAP) amplitudes, a marker of axonal loss. Identifying the molecular mechanisms causing weakness in patients with CMT1A will be important for understanding the molecular pathogenesis of the disease, as well as for future development of molecular therapies.

In order to understand further the molecular pathogenesis of CMT1A, as well as to determine which features correlate best with neurological dysfunction and clinical disability and might thus be amenable to treatment, we evaluated the clinical and electrophysiological phenotype in 42 patients with CMT1A. In these patients, muscle weakness, CMAP amplitudes and motor unit number estimates (MUNEs) correlated with clinical disability, while MNCV did not. In addition, loss of joint position sense and reduction in sensory nerve action potential (SNAP) amplitudes also correlated with disability, while sensory NCV (SNCV) did not. Taken together, these data strongly support the hypothesis that neurological dysfunction and clinical disability in CMT1A is caused by loss or damage to large calibre motor and sensory axons. Therapeutic approaches to ameliorate disability in CMT1A, as in amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases, should thus be directed towards preventing axonal degeneration and/or promoting axonal regeneration.

Methods

Patient ascertainment and evaluation

Forty-two patients with various forms of CMT were recruited from within and outside the Detroit area to be evaluated at the CMT clinic at Wayne State University. Evaluations were typically completed over a 48-h period for patients from outside the Detroit area or spread over 1 week for patients living in Detroit. Evaluations consisted of a neurological history and examination, nerve conduction study, MUNE, quantitative motor testing (QMT) and quantitative sensory testing (QST). Genetic testing through individual research laboratories, or commercially, was obtained either prior to or following the visit, to document the 1.5 Mb duplication on chromosome 17p11-p12 known to cause CMT1A. All patients gave informed consent to participating in the study, which was approved by the ethics committee of Wayne State University.

Neurological history and examination

In addition to providing a standard neurological history, patients were also asked to fill out a questionnaire designed by our group to permit entry of their neurological history into our CMT database. Among other items, the questionnaire asks patients to identify the age at which they first noted symptoms, the age at which they were diagnosed as having CMT, and to rate their disability from CMT according to a

self-assessment scale, modified from Schwab and England (Schwab and England, 1969), in which complete independence without impairment was scored at 100%, and totally bedridden and able to perform only basic bodily functions was scored at 0%.

Patients underwent standard neurological examinations. Thirty-six of the 42 patients were evaluated by a single examiner (M.E.S.) to minimize differences between examiners in grading the neurological examination. For evaluations of strength, all patients were initially examined using the traditional MRC (Medical Research Council) scale. To minimize confusion between grades 4 and 4+, or between grades <3, MRC results were altered to classify muscle strength as (1) normal (MRC grade 5); (2) mildly weak (MRC grades 4 and 4+); and (3) severely weak (MRC grades 0–3). To evaluate sensory loss in our patients, we first utilized routine neurological examinations, evaluating patients for vibratory and position sensation (large fibre modalities) and pain and temperature sensation (small fibre modalities). As was the case with motor examination, sensory grading was scored as (1) normal; (2) mildly impaired; or (3) markedly impaired. For position sensation, mild impairment was defined as the inability to detect small displacement of the great toe or fingers, and marked disability the inability to detect anything less than very large displacement of the great toe or fingers. For vibratory sensation, mild impairment was defined as a decreased threshold to vibration with a 128 Hz tuning fork in a stocking or glove distribution compared with the examiner. Marked impairment was defined as the loss of vibratory sensation to a 128 Hz tuning fork at the great toe or index finger. For pain and temperature, a mild disability was defined as a decreased threshold to pinprick or cold sensation in the toes compared with the ankles or above in the lower extremities, and a decreased threshold in the finger tips compared with the wrist or above in the upper extremities. A marked decrease to pain and temperature was defined as a decrease in pinprick or cold sensation extending to above the knees or elbows.

As part of our patient evaluation we performed an ambulation index (AI), as modified from the scale used in patients with multiple sclerosis (Hauser *et al*., 1983). Briefly, the minimum grade, 0, corresponds to normal ambulation while the maximum grade, 7, corresponds to the ability to only take a few steps, an inability to walk 25 feet and most of the time spent in a wheelchair. Our main modification of the scale was to change grade 1 from a sense of fatigue after walking 25 feet to mild gait abnormalities, such as an inverted or everted foot but not an overt steppage gait.

NCVs

NCVs were performed by standard techniques (Kimura, 1993) utilizing either Nicolet Viking or Quest machines. Temperature was maintained at 34°C. Surface electrodes were used in all studies. Sensory conduction velocities were antidromic. NCVs were calculated by standard techniques. Normals were based on laboratory determined norms and published results (Kimura, 1993). MUNEs were performed on thenar muscles innervated by the median nerve according to the technique of Daube (Daube, 1995; Yuen and Olney, 1997), utilizing the Nicolet Viking program.

QMT

QMT to measure isometric muscle contraction was performed, utilizing the BioDesign System (Vancouver, Canada), in a modified approach to that of Andres and colleagues (Andres *et al*., 1986). Testing was performed on three upper and three lower extremity muscles. For upper extremity muscles, patients were seated immediately adjacent to a standard examining table on which supports were fixed for locating a strain gauge transducer. For lower extremity testing, patients were seated in standardized positions on the table. In the upper extremity, finger extension, wrist extension and arm flexion were tested. Patients laid their forearm on a board which was flush with the end of the bed. Either their fingers, hand or wrist were inserted into a 5 cm adjustable webbed loop connected to the strain gauge, which in turn was connected to a hook on the immobile upright. The height and direction of the strap were adjusted to ensure that the pull was exactly 90° to the angle of the bed, and the tension of the strap was adjusted to ensure there was no slack in the system. In the lower extremity, foot dorsiflexion, plantar flexion and leg extension were tested. For leg extension, patients were seated with their legs hanging over the edge of the bed, flexed at 90° with the strap attached around their ankle. For foot dorsi- and plantar flexion, patients were seated with their feet on the bed, with the foot to be tested raised off the bed by a folded towel under their ankle, to prevent friction of the heel from impairing the results. The strap was placed around the foot, just at the ball of the foot, with the loop connected to the strain gauge, as was done with the upper extremity. For each test, patients were maintained in standard positions to ensure muscles were always tested in the same manner with the patient in the identical position. Force was transduced electronically by the amount of distortion of the metal ring in the strain gauge, which was coupled to an IBM compatible personal computer which displayed the force generated, in newtons, in each of the muscles tested over a period of 5 s. To ensure the patients were producing their maximum force, three independent trials for each muscle group were tested, with the patient unable to observe the screen during the study. If the runs differed by >10% from each other in maximum force generated, the trials were excluded. The maximum force generated for each muscle group was recorded. Two-thirds of the patients were evaluated by the same examiner. There were a total of three examiners involved in the study.

QST

QST was performed on all patients using a Case IV device. Studies were performed utilizing the '4.2.1' paradigm (Dyck

et al., 1993). Thresholds for vibration and cold sensation were calculated for an upper and lower extremity. Results were given in 25 discrete levels of vibratory or thermal stimuli (just noticeable difference), in age and sex matched percentiles and in μm of displacement (vibration) or °C of temperature change detected. QST was performed in a dedicated room, in standardized conditions, by one of two examiners, each of whom have demonstrated their reliability in performing the tests in inter-centre analysis of multicentre trials of sensory neuropathy.

Statistical analysis

Descriptive statistics (means and standard deviations) were calculated using the Excel statistical package. Bivariate (Pearson) correlations were calculated between variables of interest using SAS, version 6.10. The significance of all correlations (i.e. zero versus non-zero) was evaluated with $\alpha = 0.01$.

Results

Description of cohort

We have evaluated 42 patients with CMT1A identified both by their clinical and electrophysiological phenotype as well as the presence of a 1.5 Mb duplication on chromosome 17p11-p12 (Lupski and Montes de Oca-Luna, 1991; Raeymaekers *et al*., 1991). The demographic features of this group are outlined in Table 1. In general, the patients in this cohort were only mildly disabled. Most patients were ambulatory and only three patients required the use of a wheelchair. Twenty-six patients required ankle bracing and another eight used orthotics. Ten patients had undergone foot surgery, with the procedure being triple arthrodesis in seven of the cases. When asked to rate their disability using an activity of daily living scale (Schwab and England, 1969) (no impairment = 100%), the mean value of all patients was 85% (SD = 11.4%). Twenty patients graded themselves at 90–100%. Only four patients (three at 70%, one at 40%) rated themselves at scores indicating less than complete independence in all activities. Interestingly, many patients perceived that their disease had recently progressed. Fourteen patients felt they had become worse in the 12 months prior to their visit, 16 others felt they had progressed within the 6 months before their visit, while 11 felt there had been no disease progression for 24 months prior to their visit. The patient demographics, degree of disability and rate of clinical progression are similar to those of other published CMT1A cohorts (Hoogendijk *et al*., 1994; Birouk *et al*., 1997; Thomas *et al*., 1997).

Analysis of NCVs demonstrates uniform conduction slowing and a length-dependent loss of both motor and sensory axons

We evaluated NCV in both upper and lower extremities of all 42 patients with CMT1A. There are two major conclusions drawn from these studies, outlined in detail in Table 2 and Figs 1 and 2, and discussed below. First, NCVs were uniformly slowed in all nerves tested, both motor and sensory, confirming the studies of previous investigators (Kaku *et al*., 1993*a*; Hoogendijk *et al*., 1994; Birouk *et al*., 1997; Thomas *et al*., 1997) and suggesting that the demyelinating process in CMT1A is also uniform. Secondly, many CMAPs and SNAPs were also decreased. In contrast with NCVs, however, they were diminished in a variable manner, suggesting that axonal damage in CMT1A is not uniform.

NCVs of motor and sensory nerves are displayed graphically in Fig. 1. As can be seen, median MNCVs are similar to ulnar MNCVs (Fig. 1A), while median SNCVs are similar to ulnar SNCVs (Fig. 1B). In addition, comparison of the distal motor latency of the median and ulnar nerves with their sensory conductions (Fig. 2) demonstrates the similarity of sensory and motor conductions over the same nerve segment. In general, NCVs in all motor and sensory nerves were very similar in all patients, and there were only three patients with a MNCV or SNCV greater than 30 m/s in any nerve segment. Furthermore, the shape and duration of both the proximal and distal CMAPs were also similar, demonstrating the absence of conduction block or temporal dispersion (Rhee *et al*., 1990; Cornblath *et al*., 1991). Finally, terminal latency indices, another measure of the uniformity of nerve conduction, were <0.41 in all motor nerves tested (Kaku *et al*., 1994). These data, taken together, demonstrate a remarkably uniform slowing of NCV in all nerves of patients with CMT1A, suggesting that the demyelinating process in CMT1A is also uniform.

As mentioned above, CMAPs and SNAPs were decreased in many nerves in most patients. In fact, most potentials in the feet were unobtainable with surface recording. In 32 of 40 patients, for example, no peroneal CMAPs could be obtained and only three peroneal CMAPs were greater than 1 mV. In addition, only two of 39 sural SNAPs could be obtained. CMAP and SNAP amplitudes were also reduced in median and ulnar nerves of the upper extremity, though not to the same extent that we found in the longer nerves of the leg. The reduction in CMAP and SNAP amplitudes, in the absence of temporal dispersion or conduction block, most likely represents axonal loss. These data thus demonstrate the presence of a variable but length-dependent axonal degeneration of both sensory and motor nerves in most patients with CMT1A in spite of the very uniform conduction slowing. The significance of this important finding will be discussed further below.

Neurological examination of patients with CMT1A demonstrates distal weakness, atrophy and sensory loss

On clinical motor examination, muscle weakness was more pronounced in the legs than in the arms, and proximal muscles were rarely involved. In fact, weakness was found

Table 1 *Clinical information*

Age (years)	Sex (*n*)	Residence (*n*)	Foot problems (*n*)	Age at onset of symptoms (*n*)	Age at diagnosis (*n*)	Duration of disease (years)*	Disability rating (*n*)	Tremors (Roussy–Levy)	Self-reporting disease progression† (*n*)
44 ± 18.8	Male (19)	Detroit Metro Area (15)	Foot deformities (33)	0–10 years (18)	0–10 years (12)	22 ± 19	90–100% (20)	7 patients	Worse in last 12 months (14)
(range 2–79)	Female (23)	Out of Detroit Metro Area (27)	AFOs (26)	10–20 years (12)	10–20 years (9)	.	80% (16)	.	Worse in last 6 months (16)
.	.	.	Orthotics (8)	20–30 years (1)	20–30 years (2)	.	70% (3)	.	No progression in last 24 months (11)
.	.	.	Surgeries (10) (7 triple	30–40 years (2)	30–40 years (7)	.	.	.	
.	.	.	arthrodeses)	Over 40 years (5)	40–50 years (9)	.	.	.	.
.	.	.	.	Never noticed (4)	60–70 years (2)	.	.	.	.
.	.	.	.	.	Over 70 years (1)	.	.	.	.

Values for age and duration of disease are mean ± standard deviation. *This includes the time from the first noticeable symptoms to their clinic visits; five individuals diagnosed at this first visit. †Prior to their visit.

Table 2 *NCVs of patients with CMT1A*

Nerve	MNCV (m/s)		CMAP (mV)		SNCV (m/s)		SNAP (μV)		Normal values			
	n	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	Mean ± SD (range)	MNCV (m/s)	CMAP (mV)	SNCV (m/s)	SNAP (μV)
Median	37	21.3 ± 5.7 (11–35.1)	41	3.9 ± 2.9 (0–11.4)	15	20.6 ± 8.3 (9–33.3)	39	4.1 ± 10.3 (0–57)	>48	>4	>56	>25
Ulnar	36	21.5 ± 6.2 (10–34.5)	39	3.7 ± 2.3 (0–8)	17	19.6 ± 8.8 (9–36.8)	39	5.9 ± 17.2 (0–105.1)	>49	>6	>55	>10
Peroneal	8	23.9 ± 6.8 (14–32.6)	40	0.21 ± 0.65 (0–3.7)	–	–	–	–	>43	>3	–	–
Sural	–	–	–	–	2	20.5 ± 6.9 (15.6–25.4)	39	0.08 ± 0.40 (0–2.4)	–	–	>43	>6

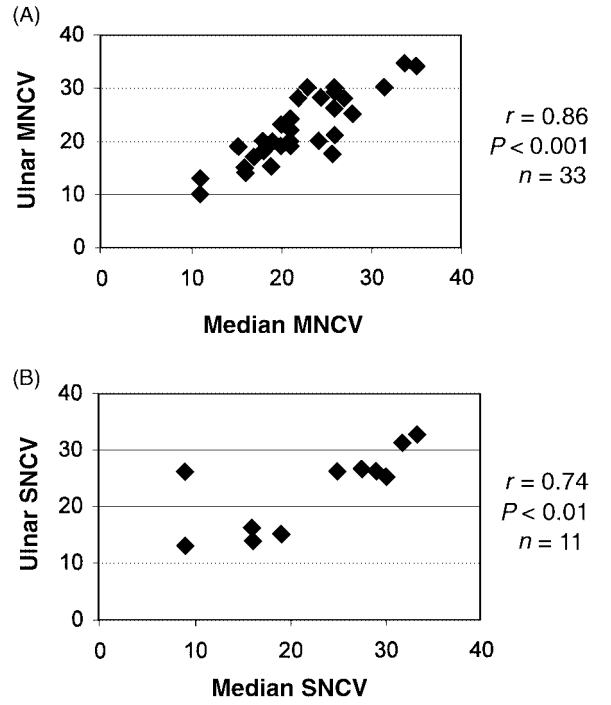

Fig. 1 Comparison of ulnar and median MNCVs (**A**) and SNCVs (**B**). The individual who has a median SNCV of 9 m/s and an ulnar SNCV of 26 m/s has carpal tunnel syndrome.

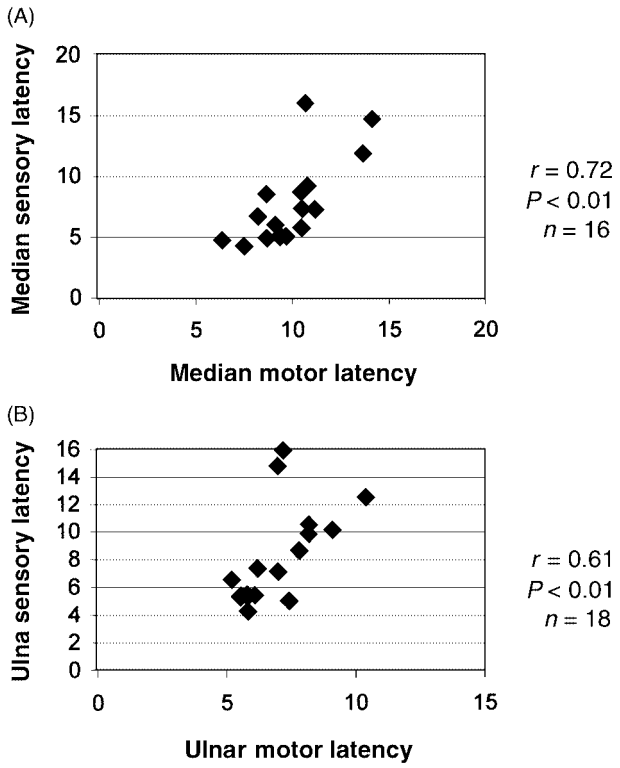

Fig. 2 Comparison of distal motor with distal sensory latencies. (**A**) Median motor latency compared with median sensory latency. (**B**) Ulnar motor latency compared with ulnar sensory latency.

almost exclusively in distal muscles. In addition, all but eight patients had evidence of distal muscle wasting in both upper and lower extremities; wasting of muscle was most pronounced where muscles were weakest. There was, however, no continuous gradient of muscle weakness or atrophy, since even patients with complete foot drop had normal proximal leg strength. Thus, when weakness became more severe, it appeared to progress from distal extensor to distal flexor muscles rather than progressing proximally up the leg or arm. These data, like the electrophysiological results, thus demonstrate predominantly distal muscle weakness and atrophy, suggesting a length-dependent degeneration of motor axons in CMT1A. Manual testing of individual muscles is summarized in Table 3.

Clinical sensory deficits, like their motor counterparts, were also more severe in the legs than in the arms and were more severe distally than proximally. The sensory deficits involved both large and small fibre modalities and were symmetrical. All patients but one had decreased vibratory sensation in the lower extremities and about half of the patients had abnormalities of position sense in their feet. In addition, all but six patients had decreased pinprick or cold sensation in a stocking distribution in the legs. Clinical sensory deficits, however, were also present in the upper extremities in about one-half of the patients. Like the motor findings, these data also demonstrate a predominantly distal sensory loss, suggesting a length-dependent degeneration of sensory axons in CMT1A. The results of the sensory examination are summarized in Table 4.

Weakness in CMT1A is a result of degeneration of motor axons, not slowed MNCVs

The above data suggest that patients with CMT1A have a length-dependent loss of motor and sensory axons, as evidenced by their reduced CMAP and SNAP amplitudes, which is associated with a uniform slowing of NCV. Since muscle weakness and atrophy and sensory loss are found in a distribution similar to that of the reduced amplitudes, these data imply that the weakness and sensory loss are a result of degeneration of motor axons, rather than slowed nerve conductions. To demonstrate this point more directly we correlated the values of the NCV, CMAP and SNAP amplitudes, measured by electrophysiological evaluation, to weakness and sensory loss measured by clinical evaluation. The results of this analysis are shown in Table 5. Median and ulnar MNCVs did not correlate with the clinical motor evaluation of the muscles they innervate. In contrast, there was a significant correlation between median and ulnar CMAPs and the clinical motor examination, $r = 0.6$ and $r = 0.5$, respectively ($P < 0.001$). Correlation analysis of peroneal MNCV and CMAP to the clinical examination could not be performed, since so many patients (33) had absent CMAPs. These data strongly support the notion that weakness in patients with CMT1A is a result of degeneration of motor

Table 3 *Manual motor testing*

Clinical motor rating	Deltoids	Biceps	Triceps	Wrist extensor	Wrist flexor	Finger flexor	Finger extensor	FDI	APB
Upper extremities (n = 42)									
Normal	41/41	41/41	41/41	39/39	40/40	37/38	8/8	9/8	11/10
Mild	1/1	1/1	1/1	3/3	2/2	5/4	31/30	20/21	20/21
Severe	0/0	0/0	0/0	0/0	0/0	0/0	3/4	13/13	11/11
Clinical motor rating	Iliopsoas	Quads	Abductors/ adductors	Hamstring	Anterior tibialis	Gastrocnemius*	EHL	Foot eversion	Foot inversion
Lower extremities (n = 42)									
Normal	40/41	37/38	40/40	39/39	10/10	25/22	3/2	13/12	18/19
Mild	2/1	4/3	2/2	3/3	22/21	10/11	19/21	13/11	14/10
Severe	0/0	1/1	0/0	0/0	10/11	6/8	20/19	15/18	9/12

All data given as left/right. Normal = MRC scale grade 5; mild = MRC scale grades 4+ and 4; severe = MRC scale grades 3 and below. FDI = first dorsal interosseus; EHL = extensor hallucis longus. *n = 41.

Table 4 *Manual sensory testing*

Clinical motor rating	Upper extremities			Lower extremities		
	Position	Vibration	Pain/temperature	Position	Vibration	Pain/temperature
Normal	30	16	18	14	1†	6
Mild	9	23	19	11	12	24
Marked	1*	1*	4	13	25	11
Normal/mild	1	1	–	–	–	1
Mild/marked	–	–	1	2	4	–

Normal/mild and mild/marked represent patients with left/right asymmetries. *Not the same patient; †4-year-old patient.

Table 5 *MNCV/CMAP versus clinical motor examination*

	n	r	P
Median MNCV versus clinical APB	37	-0.20	NS
Ulnar MNCV versus clinical FDI	36	-0.14	NS
Median CMAP versus clinical APB	41	-0.59	<0.001
Ulnar CMAP versus clinical FDI	39	-0.54	<0.001

NS = not significant; FDI = first dorsal interosseus.

Table 6 *MUNEs*

Clinical APB score	n	MUNE
Normal	5	144 ± 18 (124–170)
Mild	16	129 ± 34 (65–211)
Severe	5	91 ± 16 (73–106)

Values for MUNE are mean ± standard deviation (range). Normal = MRC scale grade 5; mild = MRC scale grades 4+ and 4; severe = MRC scale grades 3 and below.

axons, not slowed NCVs. Correlation of SNCVs or SNAPs with clinical sensory examination, however, was difficult, since only two patients had sural SNAPs and over half the patients had absent SNAPs in the hands. It is likely, therefore, that the patients' sensory loss is also a result of degeneration of sensory axons, but we are not able to demonstrate this point statistically due to the absence of SNAPs in many patients.

In order to demonstrate more directly that degeneration of motor axons is the cause of muscle weakness in patients with CMT1A, we estimated the number of motor units in the abductor pollicus brevis (APB) muscle of 26 patients using the procedure of Daube (Daube, 1995; Yuen and Olney, 1997) and correlated these results with clinical evaluation of APB weakness. These results are displayed in Table 6. Weaker patients tended to have lower MUNEs. In patients with normal APB strength (n = 5) the mean MUNE was 144 (SD = 18); in patients with mildly weak APB (n = 16) the mean MUNE was 129 (SD = 34); while in patients with severe APB weakness (n = 5) the mean MUNE was 91 (SD = 16). MUNEs, however, were difficult to obtain in patients with severe weakness. The threshold for stimulation was extremely high and reproducible CMAPs were not always possible. In addition, many patients could not keep the muscle from spontaneously twitching, which interfered with recording. For these reasons, the absolute MUNEs were often overestimated in weak patients. Supporting this explanation is the fact that in several of the patients with severe weakness, MUNEs have given much lower values in follow-up studies with a different 'technique' (Lewis *et al*., 2000). Despite these technical limitations, MUNEs correlated with both APB strength (r = 0.51, $P < 0.01$) and median CMAP amplitudes (r = 0.51, $P < 0.01$), but not with median

Table 7 *QMT*

Clinical motor rating	Arm flexion		Wrist extension		Finger extension	
	n	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	Mean ± SD (range)
Upper extremities						
Normal	37	124 ± 93 (29–456)	35	67 ± 40 (9–221)	5	35 ± 19 (19–66)
Mild	0	–	2	15 ± 7 (10–20)	28	25 ± 16 (6–89)
Severe	0	–	0	–	3	7 ± 7 (3–15)
Clinical motor rating	Leg extension		Foot dorsiflexion		Foot plantar flexion	
	n	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	Mean ± SD (range)
Lower extremities						
Normal	33	254 ± 147 (33–800)	9	80 ± 56 (22–168)	22	90 ± 47 (26–202)
Mild	3	127 ± 102 (30–234)	19	46 ± 41 (4–148)	9	67 ± 40 (28–168)
Severe	1	12.5	10	10 ± 11 (3–38)	6	24 ± 28 (2–66)

All values are given in newtons. Normal = MRC scale grade 5; mild = MRC scale grades 4+ and 4; severe = MRC scale grades 3 and below.

MNCVs ($r = 0.08$), suggesting that weakness in patients with CMT1A is due to reduced numbers of functioning motor units. Weakness in patients with CMT1A is thus a result of degeneration of motor axons.

Weakness and sensory loss both contribute to disability in patients with CMT1A

Clinical and electrophysiological evaluation of patients with CMT1A, as shown above, has demonstrated the presence of distal weakness, atrophy and sensory loss. To determine which of these clinical signs and symptoms are the major cause of patients' disability, we estimated disability using an AI in which a score of 0 indicated no disability. The average AI for our cohort was 1.8 (SD = 1.4), indicating a mildly abnormal gait but with the ability to walk 25 feet in <10 s. There was a significant correlation between AI and the patients' assessment of their own disability ($r = 0.8$, $P < 0.01$), as evaluated on a questionnaire prior to enrolment in the study. AI also correlated with the clinical evaluation of strength in the anterior tibial and extensor hallicus longus muscles of the lower extremity ($r = 0.58$, $P < 0.001$ and $r = 0.62$, $P < 0.001$, respectively), as well as with position sense in the lower extremity ($r = 0.66$, $P < 0.001$). Surprisingly, the AI also correlated with evaluation of strength in the upper extremity ($r = 0.60$, $P < 0.001$ with APB; $r = 0.52$, $P < 0.001$ with the first dorsal interosseus muscle). Thus, both motor and sensory signs contribute significantly to disability, suggesting that degeneration of sensory and motor axons is the major cause of clinical disability in patients with CMT1A.

QMT and QST of patients with CMT1A may be useful for monitoring disease progression

Both to establish a numerical baseline for longitudinal studies of CMT1A progression and to monitor patient response to potential treatment, we performed QMT and QST on our cohort of patients. Because these tests require patient co-operation, they were not performed on children of less than 5 years of age. The results of these studies, grouped by clinical examination score, are displayed in Tables 7 and 8. The weakest patients, as estimated by clinical examination, tended to have lower QMT scores, but there was considerable variability in the absolute QMT scores at each clinical grade. Correlation between the QMT score and clinical motor examination, however, was significant ($r = 0.54$, $P < 0.001$). Patients with the greatest sensory deficits also tended to have the highest QST thresholds, both for vibration (a large fibre modality) and cold (a small fibre modality), but there was variability in the absolute scores at each grade. Correlation of the QST thresholds for vibration with the clinical examination for position sense, however, was also significant in the feet ($r = 0.8$, $P < 0.001$) and hands ($r = 0.55$, $P < 0.001$), as was correlation of clinical loss of pain and temperature with the QST thresholds for cold in the feet ($r = 0.5$, $P < 0.01$) and hands ($r = 0.4$, $P < 0.01$). QMT and QST thus confirm the presence of a length-dependent loss of motor and sensory axons in patients with CMT1A and might be useful in the future, in addition to the physical examination, to evaluate progression of patients and their response to treatment.

Discussion

In this study we have demonstrated that patients with CMT1A have distal weakness, atrophy and sensory loss, which are most likely the result of a length-dependent degeneration of motor and sensory axons. In spite of the distal location of these signs and symptoms, NCVs are uniformly slowed, implying that the pathological process of demyelination is uniform. Patient weakness and sensory loss, however, are significantly correlated with decreased action potential

Table 8 *QST*

Clinical sensory rating	Vibration				Pain/temperature			
	n	JND	Percentile	Displacement (μm)	*n*	JND	Percentile	Temperature (°C)
Upper extremities								
Normal	14	10.3 ± 2.5 (4–13)	80 ± 25 (25–99)	4.6 ± 2.4 (0.4–8.3)	16	9 ± 4.2 (4–16)	79 ± 24 (40–99)	1.5 ± 1.8 (0.2–5.4)
Mild	23	12.5 ± 2.9 (7–21)	93 ± 9.7 (60–99)	14 ± 27 (1–135)	19	12.7 ± 5.7 (5–21)	92 ± 14 (50–99)	6.2 ± 8.1 (0.2–22.3)
Marked	1	21.1	99	151	4	16.8 ± 3.4 (13–20)	99 ± 0	8.8 ± 7.4 (1.9 ± 18.2)
Lower extremities								
Normal	1*	–	–	–	4	10.6 ± 4.4 (6–15)	62 ± 52 (25–99)	2.0 ± 1.9 (0.3–4.1)
Mild	12	20.1 ± 3.3 (13–25)	99 ± 1.1 (95–99)	188 ± 157 (8–99)	25	14.2 ± 5.3 (6–25)	84 ± 22 (25–99)	6.7 ± 7 (0.3–23)
Marked	26	23.3 ± 3.3 (9–25)	98 ± 3.8 (80–99)	431 ± 201 (3–577)	11	19.4 ± 3.8 (12–25)	96 ± 5.5 (80–99)	14.2 ± 7.5 (1.4–24)

Values given are mean ± standard deviation (range). JND = just noticeable difference. *QST not performed on this individual.

amplitudes in distal nerves, but not slowed NCVs, suggesting that distal degeneration of motor and sensory axons is the cause of patient disability in CMT1A.

The clinical and electrophysiological data from our study are similar to those from the classic description of CMT1 (Brust *et al*., 1978; Harding and Thomas, 1980*a*, *b*), as well as three more recent large studies of patients with CMT1A (Hoogendijk *et al*., 1994; Birouk *et al*., 1997; Thomas *et al*., 1997). Weakness was predominately restricted to distal foot or hand muscles with rare patients having proximal weakness in legs or arms (Hoogendijk *et al*., 1994). In addition, all four groups, including our own, found uniformly slowed NCVs of ~20 m/s and markedly reduced or absent CMAPs and SNAPs, particularly in the lower extremities. Taken together, these studies describe a typical ('classical') phenotype of patients with CMT1A.

Despite the similarity of our data to those of other investigators, our conclusions from these data are somewhat different, probably due to differences in the methodology used for evaluation. For example, we compared motor conductions and CMAP amplitudes with the strength of the individual muscle groups innervated by these nerves and found a significant correlation between strength and CMAP amplitudes, but not between strength and motor nerve conductions. Hoogendijk and colleagues, however, compared median motor conductions and CMAP amplitudes with a composite disability score, which included both proximal and distal muscle groups in both upper and lower extremities, muscles innervated by cranial nerves, sensory findings and reflexes, and did not find a correlation between disability and CMAP amplitudes (Hoogendijk *et al*., 1994). In addition, Birouk and colleagues compared the average MNCVs and CMAP amplitudes with a functional disability score which measured the patients' ability to walk, and likewise did not find a significant correlation between CMAP amplitudes and disability (Birouk *et al*., 1997). Because of their use of composite or averaged data, however, significant correlations between the strength of individual muscle groups, MNCVs and CMAP amplitudes might have been difficult to detect.

Demyelinating Schwann cells exert a number of adverse biological effects on their axons. Sahenk and colleagues have shown that rodent axons regenerating through nerve grafts from patients with CMT1A have a reduced calibre compared with surrounding nerves (Sahenk *et al*., 1999). In addition, de Waegh and colleagues have demonstrated that trembler nerve transplants produce local biochemical changes in axons that have regenerated through them, including alterations in neurofilament phosphorylation, increased neurofilament density and decreased axonal transport (de Waegh and Brady, 1990; de Waegh *et al*., 1992). Similar changes have also been found in nerves of patients with CMT1 (Watson *et al*., 1994). Given the nature of these biological effects, it is not suprising that patients with CMT1A have clinical and electrophysiological evidence of axonal dysfunction.

Although interaction with demyelinating Schwann cells causes abnormalities in axonal structure and function, the mechanisms by which these abnormalities cause disease in patients are not known. One obvious possibility is that weakness and sensory loss in patients are a result of axonal degeneration. This is consistent with the electrophysiological and clinical findings in patients with CMT1A and has been demonstrated to occur in several animal models of dysmyelinating peripheral neuropathy (Low and McLeod, 1975; Low, 1976; Frei *et al*., 1999; Sancho *et al*., 1999). Axonal dysfunction induced by demyelinating Schwann cells, however, could also occur without frank axonal degeneration. Decreased axonal transport of neuregulin, a peptide growth factor synthesized by neurons and required for normal trophic support of muscle, could lead to disorganization of synaptic structures and physiological dysfunction, without axonal

degeneration. In addition, Schwann cells might provide factors necessary for the normal electrophysiological properties of axons that are lacking in demyelinating nerve. Friedman and colleagues, for example, have demonstrated that demyelinating Schwann cells in Trembler mice are deficient in the expression of the trophic factors GDNF (glial derived growth neurotrophic factor) BDNF (brain derived neurotrophic factor) and NGF (nerve growth factor) (Friedman *et al*., 1999). In addition, Yuan and Ganetzky have recently identified a protein in *Drosophila*, secreted by glia and localized on axons, which is required for normal axonal excitability (Yuan and Ganetzky, 1999). Whatever the disease mechanism, axonal dysfunction in patients with CMT1A is a result of the primary process of demyelination and is the cause of the major signs and symptoms of the disease. However, the most reasonable interpretation of our results is that axonal degeneration itself is the main cause of disability in patients with CMT1A.

In summary, three previous studies as well as the current study demonstrate that CMT1A is a uniformly demyelinating disease that produces distal weakness, atrophy and sensory loss. In our current study we have further demonstrated that these clinical findings, as well as the patients' disability, are caused by a length-dependent degeneration of both sensory and motor axons. The biological basis for this apparent paradox is probably disruption of the normal interactions between axons and their Schwann cells, leading to decreased axonal function.

The fact that distal weakness and sensory loss due to a length-dependent axonal degeneration do correlate with disability in CMT1A has important implications for future treatment of the disease. Correction of the Schwann cell defect uniformly along the entire course of each affected nerve might be difficult, while correction of the Schwann cell defect distally in affected nerves might be more feasible. In fact, we have shown that adenoviral-mediated gene transfer can be used to alter Schwann cell gene expression after intraneural injection of virus for up to 30 days in immunosuppressed animals (Shy *et al*., 1995; Jani *et al*., 1999). Prevention of distal axonal degeneration in CMT1A might also be accomplished by supplying sufficient quantities of an exogenous growth factor, such as CNTF (ciliary neurotrophic factor) (Sendtner *et al*., 1992; Mitsumoto *et al*., 1994; Sahenk *et al*., 1994) or GDNF (Oppenheim *et al*., 1995; Yan *et al*., 1995) to degenerating motor and sensory axons. This approach has been used to rescue degenerating motor neurons or to stimulate their regeneration (for review, see Oppenheim, 1996) and might prevent the onset of neurological signs and symptoms if begun early in the course of the disease. CMT1A, like ALS, is thus a neurodegenerative disease.

The development of effective treatments for CMT1A, as for other neurodegnerative diseases, will also require sensitive methods for following disease progression. Killian and colleagues, for example, were unable to detect increased foot weakness over a 22-year period in a group of patients with CMT1A, even though the patients themselves felt their disease had progressed. In addition, NCV did not change with time (Killian *et al*., 1996). In our current study, we have shown that the clinical examination, in combination with electrophysiological analysis of peripheral nerve function, the AI, QMT and QST, provides a detailed picture of neuromuscular function. These techniques might prove useful for following the progression of individuals and/or groups of patients over time, as well as the response of patients to treatment. The data from our current study thus firmly place CMT1A within the framework of neurodegenerative diseases and provide methodology for assessing its response to treatment in the future.

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