



## Small fiber neuropathy in Charcot-Marie-Tooth disease

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### Abstract:

The aim of this study was to investigate small myelinated (A $\delta$ ) and unmyelinated (C) fiber function in patients with CMT1A and CMTX polyneuropathy. 17 CMT1A and 10 Cx32 polyneuropathy patients were investigated with warm and cold threshold to evaluate small myelinated (A $\delta$ ) and unmyelinated (C) somatic fiber function and with sympathetic skin responses (SSR) to evaluate post-ganglionic sympathetic fiber function.

Median age and disease duration did not differ between the two groups. Charcot-Marie-Tooth neuropathy score was higher in CMTX patients. Mean MCV differed significantly between the two groups in both Median and Ulnar nerve. In CMT1A patients warm threshold was abnormal in 72% and cold threshold in 53%. On the contrary, in Cx32 patients group warm and cold threshold was abnormal in 10 and 20% respectively. SSR was also abnormal in only a small number of both CMT1A and Cx32 patients (24% and 10% respectively).

Conclusion: Small fiber function is frequently impaired in CMT1A polyneuropathy patients.

**Key words:** Charcot-Marie-Tooth; neuropathy; small fibers.

### Introduction

Charcot-Marie-Tooth (CMT) is the most frequent inherited neuropathy. Although it is a genetically heterogeneous disorder with autosomal dominant, recessive and x-linked inheritance pattern (Shy, 2004), all symptomatic subjects present similar clinical phenotype: Progressive muscle weakness and atrophy, sensory loss, decreased or absent tendon reflexes, mainly achille's, and skeletal deformities. The most frequent form of the disease is CMT1A which results either from point mutation or duplication of peripheral myelin protein 22 (PMP22) gene on chromosome 17p11.2 (Lupski *et al.*, 1992) and represent the 70% of all CMT cases (Ionasescu, 1995). The second more frequent is the X-linked

form (CMTX) encountered in about the 20% of cases and due to mutations of connexin 32 (Cx32) gene (Bergofen *et al.*, 1993). CMT is considered a large fiber neuropathy (Dyck 1984; Sanderek *et al.*, 1999; Vital *et al.*, 2001). However some other electrophysiological and histological studies indicate that unmyelinated fibers are also impaired (Ingall *et al.*, 1991; Low *et al.*, 1978).

Nerve conduction studies (NCS) test only large diameter motor and sensory myelinated fibers, and unmyelinated fibers can be tested only with quantitative sensory tests (QST). Small fiber function has been investigated in a few cases of CMT1 and CMT2 (Hanson *et al.*, 1998; Ericson *et al.*, 1999; Lankers *et al.*, 1991), but not in CMTX polyneuropathy patients. The aim of this study was to investigate small myelinated (A $\delta$ ) and unmyelinated (C) fiber function in CMT1A and CMTX polyneuropathy patients.

### Material and methods

Seventeen patients with CMT1A (7 men and 10 women, median age  $42.4 \pm 14$  years) and ten patients with CMTX (8 men and 2 women, median age  $42.9 \pm 13.6$  years) diagnosed by DNA analysis were included in the study. All CMT1A patients presented PMP22 mutation on chromosome 17p11.2 and all CMTX patients presented mutations of Cx32 gene. Five of them were members of the same family with C.462T > G connexin 32 (Cx32) mutation. The other five patients belonged to 5 families with different Cx32 mutations. In order to compare patients of the same severity, three more female CMTX neuropathy subjects were excluded from the study because of mild involvement. The remaining two female subjects were severely affected with CMT neuropathy score (Shy *et al.*, 2005) similar to those of CMT1A patients. All subjects presented typical clinical phenotype with distal muscular weakness

and atrophy and steppage gait, distal sensory deficit and absent tendon reflexes. For disability, the Charcot-Marie-Tooth neuropathy score was used.

*Electrophysiological investigation comprised:*

1. Motor conduction velocity (MCV) of the median, ulnar, peroneal and tibial nerve and sensory conduction velocity (SCV) of the median, ulnar and sural nerve in at least one side with standard stimulating and recording techniques and a Keypoint Medronic, Sweden electromyograph. Motor and sensory distal latency, MCV, SCV, peak-to-peak amplitude of the compound muscle action potential (CMAP), and sensory nerve action potential (SNAP) amplitude from the first deflection from the baseline to the maximum negative peak were measured. 2. Electromyogram (EMG) was performed with concentric needle electrodes in the 1<sup>st</sup> dorsal interosseous and tibialis anterior muscle in all subjects. 3. Peripheral nerve small fibres were studied using quantitative sensory testing (QST): Thermal threshold (TT), and Sympathetic skin response (SSR). 3a. TT was measured using a "Triple T" device, Medelec, Old Surrey, UK, using the two alternative forced-choice method of psychophysical analysis (Jamal *et al.*, 1985). Warm and cold threshold (WT and CT) values were considered abnormal if exceeding the mean  $\pm$  2.5 SD of our control data for the corresponding age group. 3b. SSR was recorded from the left plantar surface using disc electrodes after stimulation of the right median nerve at the wrist (Shahani *et al.*, 1984). Filter band pass was 0.5-500 Hz, stimulus intensity 75 mA, stimulus duration 0.2 ms, sweep velocity 0.5 sec/div, sensitivity 0.2-0.5 mV/div. The stimuli were given at irregular intervals greater than 1 min

to avoid habituation. Latency was measured from the stimulus artifact to the first deflection from the baseline. Amplitude was measured peak-to-peak. The shorter and higher of five responses was selected. SSR was considered abnormal if absent or if it was lower and/or longer than our control values ( $x \pm 2.5$  SD). Skin temperature was maintained above 32° C.

### Statistical analysis

Statistical comparisons between the two groups were performed using the chi-square test, Fisher's exact test, Mann-Whitney U-test and unpaired t-test as indicated in table 1.

### Results

Baseline characteristics and electrophysiological findings are shown in table 1. There were 7 male and 10 female CMT1A subjects and 8 male and 2 female CMTX subjects. Mean age ( $42.4 \pm 14$  and  $42.9 \pm 13.6$  respectively) and mean disease duration ( $31.1 \pm 19.8$  and  $24.8 \pm 7.9$  respectively) did not differ between the two groups. CMT score (20 and 24 respectively) showed a statistically significant difference. Mean MCV differed significantly between the two groups in both Median and Ulnar nerves ( $p = 0.027$  and  $< 0.001$  respectively). Small fiber function was impaired in 13 (72%) CMT1A and only in 3 (30%) CMTX patients ( $p = 0.097$ ): Warm threshold was abnormal in 9 (53%) CMT1A and only in 1(10%) Cx32 subjects. Cold threshold was abnormal in 13(72%) CMT1A and also only in 2(20%) Cx32 subjects. As to postganglionic

Table 1

Demographic and electrophysiological data of CMT1A and CMTX patients

Characteristics	CMT1A (N = 17)	CMTX (N = 10)	p
Male*	7 (44%)	8 (80%)	0.456
Age, years (mean, SD)	$42.4 \pm 14.0$	$40.3 \pm 13.5$	0.917
Disease duration, years (mean, SD)	$31.1 \pm 19.8$	$24.8 \pm 7.9$	0.373
CMT score, points (median, interquartile range)#	20 (4.5)	24 (2.5)	0.017
MMCV (mean, SD-m/sec)***	$20 \pm 6$	$28 \pm 9$	0.027
UMCV (mean, SD-m/sec)***	$19 \pm 5$	$35 \pm 9$	< 0.001
QST**	13 (72%)	3 (30%)	0.097
WT**	9 (50%)	1 (10%)	0.091
CT**	13 (72%)	2 (20%)	0.019
SSR**	4 (24%)	1 (10%)	0.621

\*  $\chi^2$ -test \*\* Fisher's exact test. \*\*\*Unpaired t-test # Mann-Whitney U-test.

Statistical comparisons between the two groups were performed using chi-square test, Fisher's exact test, Mann-Whitney U-test and unpaired t-test as indicated.

MMCV: Median motor nerve conduction velocity, UMCV: Ulnar motor nerve conduction velocity, QST: Quantitative sensory testing, WT: Warm threshold, CT: Cold threshold, SSR: Sympathetic skin response.

sympathetic nerve dysfunction, no significant difference between the two groups was observed: SSR was abnormal in 4 (24%) CMT1A patients and in 1(10%) CMTX patients ( $p = 0.653$ )

### Discussion

The aim of this study was the investigation of peripheral small nerve fiber function in the two major subgroups of CMT neuropathy, confirmed by DNA analysis, CMT1A and CMTX, by quantitative determination of somatosensory thresholds and investigation of postganglionic unmyelinated, sympathetic nerve fibers.

In order to achieve comparable types of CMT, we compared mean age of the patients and mean disease duration which did not differ significantly between the two groups. Disease severity (CMT score) was higher in CMTX patients. Regarding the involvement of small fibers, a significant difference between the two groups was observed: CMT1A patients presented impaired small fiber function significantly more frequently than Cx32 polyneuropathy patients. The second finding is the significant difference regarding MCV, similar to the reported in other studies (Anzini *et al.*, 1997; Rouger *et al.*, 1997). As to postganglionic sympathetic nerve dysfunction, was not observed a significant difference between the two groups. Small myelinated (A $\delta$ ) nerve fibers were found involved in 72% and nonmyelinated (C) nerve fibers in 53% of CMT1A patients while in Cx32 polyneuropathy patients both A $\delta$  and C nerve fiber function was impaired in only 10-20%. Most histopathologic studies show severe lesion of myelinated and intact unmyelinated fibers (Behse *et al.*, 1977; Dyck, 1984). Vital *et al.* (2001) performed a superficial peroneal nerve biopsy in 8 CMTX patients and mild lesions of unmyelinated fibers were found in only one of them (12.5%). Behse *et al.* (1977) also reported reduced number on unmyelinated nerve fibers in only 3 out of 21 CMT patients (14.3%). On the contrary, Low *et al.* (1978) found abnormalities of unmyelinated fibers in the sural nerve in patients with CMT1. In nerve biopsies the lesser diminution in the number of small fibers could be at least in part due to signs of regeneration given that nerve sprouts are frequently considered as small nerve fibers (Behse *et al.*, 1978; Dyck 1984).

Small nerve fiber function has been studied in a few small series of CMT1A and CMT2. Hanson *et al.* (1998) using thermal threshold and SSR, found normal warm threshold and abnormal cold threshold in 5 CMT1 patients tested and prolonged SSR latency in 2 of them, a finding indicating disturbed small myelinated A $\delta$  and intact C fibers. Disturbed

A $\delta$  fibers were also found by Lankers *et al.* (1991) in a CMT1 polyneuropathy patient. Ericson *et al.* (1999) studied 10 patients with CMT1 neuropathy (five of them with CMT1A) and 10 with CMT2 neuropathy and found abnormal cold threshold in CMT1 and abnormal warm threshold in both groups. Ingall *et al.* (1991) found abnormal sweat tests on the extremities of 4 CMT patients tested, a finding consistent with distal degeneration of sympathetic nerve fibers. Low efferent conduction velocity of the SSR was observed in 10 CMT patients by Denislic *et al.* (1993).

PMP22 and Cx32 are both peripheral nervous system myelin proteins, the former being one of the main structural proteins in compact myelin, and the last a gap junction protein in the non-compact domain of the myelin. CMT1A is characterized by marked slowing of MCV and SCV (Dyck 1984), while CMTX is also a demyelinated neuropathy (Anzini *et al.*, 1997; Scherer *et al.*, 1998), but is characterized by mild slowing of CV (Anzini *et al.*, 1997; Rouger *et al.*, 1997).

We can attribute the higher percentage of small fiber involvement in CMT1A in contrast to Cx32 patients, to secondary lesion of these fibers, because of the greater impairment of the myelin sheath in the former as it results from the very low CVs in CMT1A and the mild reduction of CVs in Cx32 polyneuropathy patients. Although disease severity (CMT score) was higher in CMTX patients small fibers were less frequently involved. We can postulate that the greater reduction of CVs is associated with more frequent involvement of small fibers. It is known that is not easy to compare CMT patients at the same stage of severity. In order to achieve comparable types of CMT, we compared mean age of the patients and mean disease duration which did not differ significantly between the two groups.

In conclusion, in this study peripheral somatic small nerve fiber function was found to be impaired in a significant number of CMT1A patients. The involvement of these fibers in CMTX patients was found significantly lower, as well as the involvement of postganglionic sympathetic nerve fibers in both groups.

### REFERENCES

- Shy ME. Charcot-Marie-Tooth disease:an update. *Curr Opin Neurol.* 2004;17:579-582.
- Lupski JR., Garcia CA. Molecular genetics and neuropathology of Charcot-Marie-Tooth disease type I. *Brain pathol.* 1992;2:337-349.
- Ionasescu VV. Charcot-Marie-Tooth neuropathies: From clinical description to molecular genetics. *Muscle Nerve.* 1995;18:267-275.

- Bergofen J, Scherer SS, Wang S, Scott MO, Bone LJ. *et al.* Connexin mutations in x-linked Charcot-Marie-Tooth disease. *Science*. 1993;262:2039-2042.
- 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 Neuropathies* Philadelphia, Saunders, 1984, pp 1600-1655.
- Sanderek J, Hermanns B, Bergmann C, Boroojerdi B, Bajbouj M. *et al.* X-linked dominant Charcot-Marie-Tooth neuropathy: clinical, electrophysiological, and morphological phenotype in four families with different connexin32 mutations (1). *J Neurol Sci*. 1999;167:90-101.
- Vital A, Ferrer X, Iagueny A, Vandenberghe A, Latour P. *et al.* Histopathological features of X-linked Charcot-Marie-Tooth disease in 8 patients from 6 families with different connexin32 mutations. *J Periph Nerv Syst*. 2001;6:79-84.
- Ingall TJ, McLeod JG. Autonomic function in hereditary motor and sensory neuropathy (Charcot-Marie-Tooth disease). *Muscle Nerve*. 1991;14:1080-1083.
- Low PA, McLeod JG, Prineas JW. Hypertrophic Charcot-Marie-Tooth disease. Light and electron microscope studies of the sural nerve. *J Neurol Sci*. 1978;35:93-115.
- Hanson P, Deltombe T. Preliminary study of large and small peripheral nerve fibers in Charcot-Marie-Tooth disease, type I. *Am J Phys Med Rehabil*. 1998;77:45-48.
- Ericson U, Borg K. Analysis of sensory function in Charcot-Marie-Tooth disease. *Acta Neurol Scand*. 1999;99:291-296.
- Lankers J, Frieling A, Kunje K, Bromm B. Ultralate cerebral potentials in a patient with hereditary motor and sensory neuropathy type I indicate preserved C-fibre function. *J Neurol Neurosurg Psychiatr*. 1991;54:650-652.
- Shy ME, Blake J, Krajewski K, Fuerst DR, Laura M. *et al.* Reliability and validity of the CMT neuropathy score as a measure of disability. *Neurology*. 2005;64:1209-1214.
- Jamal GA, Hansen S, Weir AI, Ballantyne JP. An improved automated method for the measurement of thermal thresholds. I. Normal subjects. *J Neurol Neurosurg Psychiatr*. 1985;48:354-360.
- Shahani BT, Halperin JJ, Boulou P, Cohen J. Sympathetic skin response – a method of assessing unmyelinated axon dysfunction in peripheral neuropathies. *J Neurol Neurosurg Psychiatr*. 1984;47:536-542.
- Anzini P, Neuberg DH-H, Schachner M, Nelles E, Willecke K. *et al.* Structural abnormalities and deficient maintenance of peripheral nerve myelin in mice lacking the gap junction protein connexin32. *J of Neurosci*. 1997;17:4545-4551.
- Rouger H, LeGuern E, Birouk N, Goudier R, Tardieu S. *et al.* Charcot-Marie-Tooth disease with intermediate motor conduction velocities: Characterization of 14 Cx32 mutations in 35 families. *Human Mut*. 1997;10:443-452 .
- Behse F, Buchthal F. Peroneal muscular atrophy (PMA) and related disorders. II. Histological findings in sural nerves. *Brain*. 1977;100:67-85.
- Behse F, Buchthal F. Sensory action potentials and biopsy of the sural nerve in neuropathy. *Brain*. 1978;101:473-493.
- Denislic M, Trontelj JV. Autonomic function in HMSN: Evidence of slowed sudomotor conduction. *Muscle Nerve*. 1993;16:114-115.
- Scherer SS, Xu YT, Nelles E, Fischbeck K, Willecke K. *et al.* Connexin32-null mice develop demyelinating peripheral neuropathy. *Glia*. 1998;24:8-20.

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