

Slowing of central conduction in X-linked Charcot-Marie-Tooth neuropathy shown by brain stem auditory evoked responses

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Abstract

Background—The most common form of CMT with slow nerve conduction velocities (CMT type I) is CMT1A, caused by a submicroscopic duplication of a region of DNA on chromosome 17 including the *PMP22* gene. This gene is expressed in peripheral nerve but not in the CNS. The second most common form is CMTX, caused by mutations in the *connexin32* gene in the X chromosome. *Connexin32* is expressed both in brain and in peripheral nerve. These molecular variants are difficult to distinguish clinically.

Methods—Brain stem auditory evoked responses (BAERs) were measured in patients with CMTX and CMT1A.

Results—BAERs showed central conduction slowing in male patients with CMTX which did not overlap the normal range. Patients with CMT1A had a delay in wave I latency but otherwise normal responses. These results are consistent with the pattern of expression of *PMP22* in the peripheral portion of the eighth nerve (myelinated by Schwann cells) and of *connexin32* in the central portion in the brainstem auditory pathways (myelinated by oligodendrocytes). This is the first evidence for central involvement in CMTX.

Conclusion—BAERs are useful to distinguish CMTX from CMT1A and may assist selection of appropriate patients for *connexin32* mutation analysis.

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Charcot-Marie-Tooth (CMT) neuropathy is the commonest group of genetic disorders of peripheral nerve with prevalence estimates ranging from one in 3500 to 8000 population.¹⁻³ There are two subgroups of CMT according to nerve conduction velocity. That with slow motor nerve conduction velocities (hereditary motor and sensory neuropathies type I, HMSN I) is more common than CMT type II (HMSN II), an axonal disorder with relatively normal conduction velocities.⁴ Molecular genetic techniques have recently shown that CMT type I is heterogenous and includes disorders caused by abnormalities in Schwann cell myelin proteins.⁴⁻⁵ The most common variety of CMT type I is CMT1A.

Some lines of evidence suggest that CMT1A is due to an overdose of the peripheral myelin protein, PMP22, resulting from a duplication of the region of DNA including the *PMP22* gene.^{6,7} CMT1B is rare and is caused by mutations in myelin protein zero (Po).⁸ Both the PMP22 and Po proteins are expressed in compact myelin.

The next most common form of CMT type I after CMT1A, has a locus on the X chromosome (X-linked CMT, CMTX) and has recently been shown to be caused by mutations in the gap junction gene *connexin32*.⁹ This may account for 10% to 20% of the total number of type I families.⁴ No single test can distinguish CMTX from other varieties of CMT type I. Possible CMTX families can be recognised if there is no male to male inheritance of the disease and when affected patients have no chromosome 17 DNA duplication. Additional evidence towards a diagnosis of CMTX is provided if intermediate range motor nerve conduction velocities are found in female CMT carriers.¹⁰ This is not always possible as males with CMTX may present as isolated or sporadic cases of CMT when the female carrier mother is asymptomatic. As it is difficult and expensive to confirm the presence of CMTX *connexin32* mutations by DNA sequencing, there is a need for further clinical tests to accurately define those with CMTX.

A total of 33 different mutations have now been described in the gap junction protein *connexin32* in 39 unrelated families with CMTX.¹¹ The protein is expressed in several tissues¹² including glia and neurons in the CNS. *Connexin32* is part of a family of gap junction proteins and may have structural and pore properties.¹³ In peripheral nerve *connexin32* is found in Schwann cell membranes in the nodes of Ranvier and in the Schmidt-Lantermann incisures but not in compact myelin.⁹

Connexin32 transports small molecules up to 1000 Da. Such small molecules may have a nutritional or messenger function. When three CMTX mutations were expressed in a paired oocyte expression system, loss of junctional conductance occurred showing that *connexin32* mutations may destroy pore function.¹⁴

As *connexin32* is expressed in the CNS in both glia and neurons and as some of our patients had mild deafness, we tested hearing and brain stem auditory evoked responses (BAERs) in 10 male patients with CMTX, to determine if auditory pathways are affected in CMTX. Female patients with CMTX were

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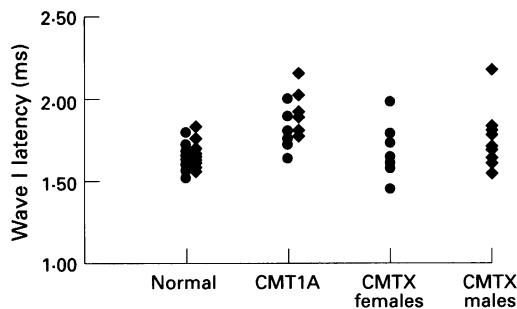


Figure 1 Wave I brain stem auditory evoked response latencies. Latency (ms) of wave peak 1 in normal male patients (10) and female patients (9), CMT1A male patients ($n = 10$) and female patients ($n = 7$), and CMTX male (10) and female ($n = 8$) groups; see table for statistics.

included as a separate group in this study as the disease is variably expressed in females and they are often only mildly affected. This is due to the effects of lyonisation (variable X chromosome inactivation).¹⁰ The BAERs in patients with CMTX were compared with those in patients with CMT1A and those in normal, unaffected people.

Methods

PATIENT SELECTION

Consecutive patients with CMTX and CMT1A presenting to the Concord Hospital CMT clinic for review were invited to participate in this study. Each was questioned about the presence of deafness. Patients with CMTX or CMT1A were defined by the presence of *connexin32* mutations or chromosome 17 duplications respectively.

CMT MUTATIONS

Connexin32 mutations were detected by DNA sequencing of double stranded polymerase chain reaction products as described by Bergoffen *et al.*⁹ The following CMTX mutations were detected: Pro 158 Ala, Val 35 Met, Ser 182 Thr, deletion 110–116,¹⁵ Arg 220 to stop, 1 base pair deletion base 158 giving stop at 195. The CMT1A duplication was determined with probe VAW409R3 (D17S122) and densitometry of autoradiographs⁶ and pulse field gel analysis.¹⁶

BRAIN STEM AUDITORY EVOKED RESPONSE STUDIES

The BAERs were elicited with monaural click stimuli of 0.1 ms duration with both rarefaction and condensation polarity and were recorded on a Medlec Sensor. Click hearing threshold was determined and a stimulus intensity of 70 dB above threshold was used. The right and left ears were stimulated independently with the unstimulated ear masked with white noise at an intensity of 30 dB. Surface electrodes were used with the reference electrode placed at Cz and the active electrodes placed on the ear lobes at A1 and A2. Click stimuli were delivered at a rate of 10 Hz. The BAERs were recorded with a low frequency filter of 3 Hz and a high frequency filter of 3 kHz. Analysis time of 10 ms after the

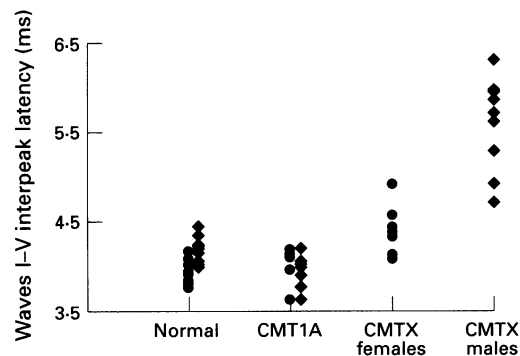


Figure 2 Wave I-V interpeak brain stem auditory evoked response latencies. Interwave latency (ms) between wave peaks 1 and 5 in the groups shown in fig 1 (normal patients, CMT1A, and CMTX male and female groups); see table for statistics.

stimulus was used and 1024 responses averaged with two runs of both polarities recorded for each ear. The latencies for peaks of waves 1 to 5 were measured for further analysis.

Results

SYMPTOMATIC DEAFNESS

One woman with CMT1 (aged 77) and two men with CMTX (aged 70 and 35) had hearing loss on questioning. The 35 year old was slightly deaf in one ear (-25 dB at 6 kHz) and had difficulty hearing in a crowd of people. Audiograms detected mild high frequency sensorineural deafness in these three patients, which was insufficient to prevent BAERs.

BRAIN STEM AUDITORY EVOKED RESPONSE STUDIES

Control values for BAERs were obtained from 19 normal subjects ranging in age from 22 to 51 (mean 34.6) years. The 17 patients with CMT1A ranged in age from 17 to 77 (mean 42.9) years and the 10 male patients with CMTX were aged 12 to 70 (mean 34.3) years. Eight female patients with CMTX were aged 11 to 78 (mean 37.4) years. Satisfactory BAER waveforms were obtained for all subjects. Waveforms were not attenuated in either patients with CMT1A or those with CMTX.

The mean click threshold for the 19 control subjects was 11.97 (SD 3.95) dB, the 17 patients with CMT1A 15.88 (SD 15.49) dB, the male patients with CMTX 9.25 (SD 7.99) dB, and the female patients with CMTX 14.38 (SD 7.99) dB. The click thresholds for male and female patients with CMTX were not significantly different from controls.

Wave I latencies were significantly longer in patients with CMT1A compared with controls (table) suggesting delayed conduction in the distal eighth nerve. Later BAER wave latencies and interwave latencies beyond wave I for CMT1A were not significantly different from controls (table).

Wave I latencies for patients with CMTX were not significantly different from controls, unlike the patients with CMT1A (table). Later waveforms beyond wave I and I-V interwave latencies were all significantly delayed in the

Mean (SD) BAER wave peak latencies (ms)

Latency	Normal subjects	CMT1A	CMTX males	CMTX females
Wave I	1.65 (0.08)	1.85 (0.13) P < 0.001	1.74 (0.18)	1.67 (0.16)
Wave II	2.78 (0.11)	2.90 (0.16)	3.16 (0.21) P < 0.000	2.28 (0.22)
Wave III	3.82 (0.14)	3.98 (0.19)	4.37 (0.22) P < 0.000	3.89 (2.13)
Wave IV	4.96 (0.15)	5.13 (0.20)	5.99 (0.45) P < 0.001	5.13 (0.17) P < 0.05
Wave V	5.17 (0.18)	5.85 (0.25)	7.25 (0.51) P < 0.001	6.03 (0.29) P < 0.01
Interwave I-III	2.17 (0.13)	2.13 (0.13)	2.64 (0.14) P < 0.001	2.22 (0.12)
Interwave III-V	1.89 (0.13)	1.87 (0.16)	2.88 (0.45) P < 0.001	2.14 (0.19) P < 0.001
Interwave I-V	4.06 (0.17)	4.00 (0.18)	5.51 (0.53) P < 0.001	4.36 (0.28) P < 0.01

Mean latencies for right and left ears using rarefaction and condensation click stimuli in normal subjects (10 male and nine female patients), patients with CMT1A (10 males and seven females) and patients with CMTX (10 males and eight females). P values are for two tailed independent sample *t* tests comparing the CMT groups with the control group of the appropriate sex.

patients with CMTX relative to the patients with CMT1A and control subjects (table). The findings in male and female patients with CMTX indicate slowing of conduction in central auditory pathways beyond the distal eighth nerve and indicate that slowing of conduction is seen to the level of the upper pons. The difference was less in female patients with CMTX and values overlapped the normal range. There was no overlap for waves I-V interwave latency between the CMTX male group and the CMT1A and control groups. The longest mean wave I-V interwave latency in the control group was 4.43 ms; the longest CMT1A wave I-V interwave latency was 4.18 ms; and the shortest CMTX wave I-V interwave latency was 4.71 ms.

Discussion

Central delay and increased interpeak latencies were found in all male patients with CMTX. Wave I delay was restricted to patients with CMT1A. The results are therefore in accord with the pattern of expression of the *PMP22* and *connexin32* genes. Further, these results provide some evidence that the wave I generator is situated central to peripheral myelin and that the *connexin32* gene is expressed in the proximal acoustic nerve and its central connections. The suggested localisation of the generator sources for the waveforms of the BAER in humans are wave 1, distal eighth nerve; wave 2, proximal eighth nerve or cochlear nucleus; wave 3, lower pons; wave 4, mid-pons or upper pons; wave 5, upper pons or inferior colliculus.¹⁷

The conduction slowing found in CMTX was in the central auditory pathway, which is sheathed by oligodendroglial myelin. Another test of central conduction—somatosensory evoked responses—could not be reliably obtained due to the peripheral neuropathy obscuring the central responses and were, therefore, not included in the study.

Previous investigators have reported varied BAER abnormalities in patients with CMT type I, some with wave I delay^{18,19} and others with interpeak delays²⁰⁻²² or either wave I or

interpeak delays.²³ Patients with both wave I delay and interpeak delay were reported by Garg *et al.*²⁴ All these studies were performed before it was possible to separate the two common forms of HMSN I—CMT1A and CMTX.

In these earlier studies dominant inheritance was not strictly defined by the presence of male to male inheritance and some multi-generation families without male to male inheritance which could be X-linked families were included. Interpeak BAER delays were probably due to unrecognised cases of CMTX.

We had two patients with both wave I delay and increased interpeak latency indicating that both central and peripheral slowing may occur in CMTX. This is to be expected as considerable slowing of nerve conduction occurs in CMTX but on average is 10 m/s faster than in CMT1A.

Our results for CMT1A are similar to those reported in CMT type I by Scaioli *et al.*,¹⁹ who found delay in wave peak I consistent with slowing of conduction in the peripheral acoustic nerve. Delay in wave I has also been reported in demyelination in the Guillain-Barré syndrome.²⁵

Raglan *et al.*²² thought that BAER abnormalities explain hearing loss in HMSN I but our results showed that all our male patients with CMTX had interpeak delays but only two had symptomatic hearing loss and mild high frequency loss on audiograms. One had mild unilateral high frequency loss and the other, like the severely affected patients of Raglan *et al.*,²² was an older patient. This patient and the patient with CMT1A with hearing loss were the two oldest members of the study.

Interpeak delay may be present from birth as even patients as young as 8 years have delays as prominent as older subjects. Therefore, slowing of central conduction may be analogous to the lifelong slowing of nerve conduction in CMT1A^{26,27} and deafness is an age dependent central phenomenon equivalent to the age dependent decrease in motor action potential amplitude in CMT1A.²⁶

This study is the first evidence of central conduction delay and auditory pathway abnormalities in CMTX and further detailed studies of other central functions are warranted. The delay was unrelated to hearing loss. No central involvement has yet been found in CMT1A. The pathophysiological basis of these findings requires further investigation and suggests a central myelination defect and possible axonal effects in CMTX.

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