

## SHORT REPORT

## Six novel connexin32 (GJB1) mutations in X-linked Charcot-Marie-Tooth disease

M-J Lee, I Nelson, H Houlden, M G Sweeney, D Hilton-Jones, J Blake, N W Wood, M M Reilly

*J Neurol Neurosurg Psychiatry* 2002;**73**:304–306

X-linked Charcot-Marie-Tooth disease (CMTX) is a clinically heterogeneous hereditary motor and sensory neuropathy with X-linked transmission. Common clinical manifestations of CMTX, as in other forms of Charcot-Marie-Tooth disease (CMT), are distal muscle wasting and weakness, hyporeflexia, distal sensory disturbance, and foot deformities. Motor nerve conduction velocity is reduced. In male patients it is often less than 38 m/s in the median nerve (a value often used to distinguish between "demyelinating" and "axonal" forms of CMT), but in female patients conduction velocity may be faster than this or normal. Mutations in the connexin32 (gap junction protein  $\beta$  1 (GJB1)) gene are responsible for the majority of CMTX cases. This report describes six British CMTX families with six novel mutations (four missense, one nonsense, and one frame shift) of the GJB1 gene. Affected members in these six families had typical signs of CMT but in some affected members of three families there was additional central nervous system involvement or deafness in the absence of any other explanation other than CMT.

Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogeneous hereditary motor and sensory neuropathy characterised by distal muscle wasting and weakness, sensory disturbance, hyporeflexia, and foot deformities. It is a common disorder with a population frequency of about 1:2500.<sup>1</sup> CMT is broadly classified into type 1 (demyelinating, median motor nerve conduction velocity (MCV) < 38 m/s) and type 2 (axonal, MCV > 38 m/s).<sup>2</sup> The most common mode of inheritance for both type 1 and type 2 is autosomal dominant but some CMT families have X-linked inheritance (CMTX). CMTX patients have features of both type 1 and type 2, although some have mild central nervous system (CNS) involvement.<sup>3–4</sup> Results of electrophysiological studies in patients with CMTX are variable with MCVs in affected males usually either in the demyelinating range or in the intermediate range. Females are usually more mildly affected with MCVs that can be either in the intermediate or in the axonal range, or can be normal.<sup>5–9</sup> Undue reliance on neurophysiological findings in female patients may lead to a delay in diagnosis, especially if they are in the axonal range and a family member has been given a misdiagnosis as probable autosomal dominant CMT type 2.

The connexin32 (gap junction protein  $\beta$  1, GJB1) gene is one of the genes responsible for CMTX.<sup>10–12</sup> To date 230 mutations of GJB1 gene have been reported to cause CMTX (the Inherited Peripheral Neuropathies Mutation Database is available at <http://molgen-www.uia.ac.be/CMTMutations/>). Mutations of the GJB1 gene are widely distributed over the entire gene. In this report, we describe the clinical and electrophysiological features of six patients with CMTX, in whom we identified six novel GJB1 mutations.

## PATIENTS AND METHODS

## Patients

Six families with inherited neuropathy were studied. All families originated from the south of England. Ethical approval for genetic studies in neuropathy had been obtained from the National Hospital for Neurology and Neurosurgery Ethics Committee.

Table 1 presents the clinical and electrophysiological details. All index cases of the disease had been diagnosed as probable CMT based on typical clinical features of distal wasting, weakness, hyporeflexia with distal sensory disturbance in most patients, and a positive family history. In five index cases the chromosome 17 duplication had been excluded previously. In the sixth index case the median MCV was 32 m/s and a nerve biopsy suggested an axonal neuropathy, so GJB1 was thought to be the most likely cause and was screened for first. The first five index cases were chosen for GJB1 mutation screening because the chromosome 17p11.2 duplication was negative and the electrophysiological findings were compatible with CMTX (demyelinating/intermediate in males, axonal/intermediate in females) and there was no male to male transmission in any family.

## METHODS

DNA was extracted from leucocytes using a standard phenol-chloroform method.<sup>13</sup> The coding region of the GJB1 gene was amplified using primers reported by Bergoffen *et al.*<sup>10</sup> The annealing temperature for polymerase chain reaction was 56°C to 53°C in a touch down protocol (that is, reduced 0.1°C per cycle) for 25 cycles and then was held constant at 53°C for 12 cycles. The sequencing reactions were carried out using the polymerase chain reaction primers and BigDye Terminator cycle sequencing chemistry (Applied Biosystems, Foster City, California, USA). Sequences were analysed on an ABI377 automated DNA sequencer (Applied Biosystems).

## RESULTS

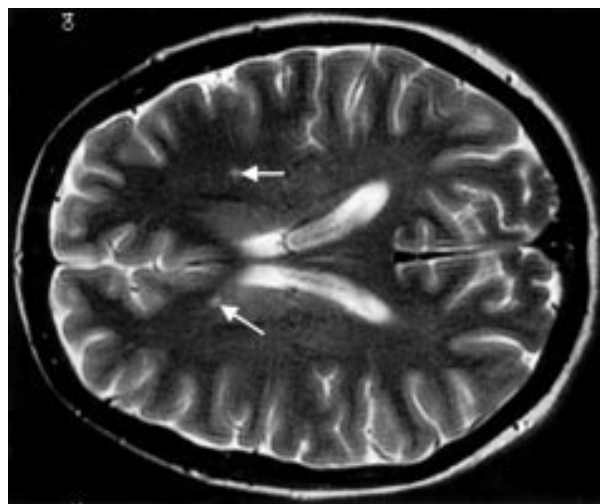
Direct sequencing of the GJB1 gene in these six patients identified six novel mutations. As table 1 shows, the six mutations were four missense, one nonsense, and one frame shift mutation. Both W24C (missense) and T55R (missense) are highly conserved amino acids among GJB1 and other gap junction protein sequences (such as GJB3 and GJB4) in *Xenopus laevis*, chicken, and all mammals. V125D (missense) and F153S (missense) are also highly conserved in the GJB1 proteins among all mammalian species. These mutations were not present in 50 normal controls. In families 1, 3, and 5, DNA

**Abbreviations:** CMT, Charcot-Marie-Tooth disease; CMTX, X-linked Charcot-Marie-Tooth disease; CNS, central nervous system; GJB1, gap junction protein  $\beta$  1; MCV, motor nerve conduction velocity; MRI, magnetic resonance image

**Table 1** Clinical manifestations and electrophysiological studies of the families with gap junction protein  $\beta 1$  (GJB1) mutations

Family	Mutation	Patient	Clinical manifestations			Electrophysiology						
			Age at onset (years)	Sex/age (years)	Muscle wasting (upper/lower)	Sensory disturbance	Tendon reflex	CNS involvement	Ulnar MCV (m/s)/DML (ms)	Ulnar CMAP (mV)	Median SAP	Sural SAP
1	W24C	1	15	F/30	D>P, +/+++	Large fibre, mild	Hyporeflexia	Extensor plantar response	53/2.7	8.0	3.5	Absent
		2	11	F/28	D>P, +/+++	Large fibre, mild	Absent AJ		24/3.6	0.1	Absent	
		3	12*	M/66	D>P, +/+++	Both, severe	Areflexia	Deafness and abnormal auditory brainstem potentials	33/4.9	2.5	Absent	
2	T55R	4	16*	M/71	D>P, +/+++	Both, moderate	Areflexia	Brain MRI: high signal in the cerebral hemisphere and right mid brain in T2WI	49/3.8	0.72		
		5	25	F/33	D>P, +/+++	Large fibre, mild	Areflexia					
3	E109Stop	6	4*	M/12	D>P, +/+++	No	Areflexia		37/3.4	1.2		16.1 (right medial plantar)
		7	15 months	M/5	D>P, +/-	No	Areflexia		49/2.3	2.4		Absent
4	V125D	8	9*	M/16	D>P, +/+++	Large fibre, mild	Hyporeflexia, absent AJ	Deafness and extensor plantar response	37/4.5	3.5	Absent	
		9	9*	M/12	D>P, +/-	No	Normal, brisk AJ		51/2.9	12.8	11.3 (right radial)	Absent
5	F153S	10	F/42	Asymptomatic				55/2.6	15.0	Absent		
		11	12	M/52	D>P, +/+++	Both, moderate	Hyporeflexia		44.7/3.2	6.8	Absent	
6	T191Fs	12	16*	M/54	D>P, +/+++	Large fibre, severe, small fibre, moderate	Areflexia		33/4.6	1.7	Absent	Absent

±, minimal; +, mild; ++, moderate; +++, severe; AJ, ankle jerks; CMAP, compound motor action potentials; CNS, central nervous system; D, distal; DML, distal motor latency; F, female; Fs, frame shift; M, male; MCV, motor conduction velocity; MRI, magnetic resonance image; P, proximal; SAP, sensory action potential, T2WI, T2 weighted image. \*Index case.



**Figure 1** Magnetic resonance image of the brain of patient 5, family 3. There are non-specific but definitely pathological white matter lesions (arrows).

from other affected members was available and the appropriate mutation was confirmed in each affected patient.

Clinically, the affected patients were similar to previously described patients with CMT who had GJB1 mutations, with a predominantly distal motor and sensory neuropathy in most patients (table 1). Males were more severely clinically affected and had slower MCVs than females. Interestingly, affected members from four families had CNS involvement or deafness, as has been previously described with GJB1 mutations.<sup>3-4</sup> In family 1, one affected female (patient 1, table 1) had bilateral extensor plantars but this was probably explained by a second diagnosis of spastic diplegia secondary to prematurity. In family 2, patient 4 had deafness and abnormal auditory brainstem potentials, and in family 6, patient 12 also had deafness and extensor plantars with no other explanation other than CMT. Patient 5, family 3 had an interesting magnetic resonance image (MRI) of the brain (fig 1) with mild but definitely abnormal white matter high signal lesions for her age without any other explanation other than CMT.

## DISCUSSION

CMTX is a common cause of inherited neuropathy. We report six novel mutations of the GJB1 gene from six British CMTX families.

The disease onset of the six index patients was usually in later childhood or adolescence with one notable exception. Patient 7, family 3 was noted to have difficulty walking at age 15 months but also had ligamentous laxity. At the age of three years and 10 months his reflexes were present but by age five and a half he was clinically affected with absent reflexes and high arches. He tolerated only limited nerve conduction studies at age five but his ulnar MCV at this stage was 49 m/s. Similarly, patient 9, family 5 was definitely affected but his ulnar MCV at age 12 was 51 m/s. Unfortunately, median MCV was not available.

The clinical features of the neuropathy in our patients were similar to those reported previously in patients with GJB1 mutations, including more severe involvement in affected males than in females. Electrophysiological findings fit well with other studies. All patients had motor and sensory involvement electrically, with affected males having upper limb MCVs in the intermediate or demyelinating range (except for the two exceptions mentioned), while affected females tended to have MCVs in the axonal range.

We did find a high incidence of mild CNS involvement and deafness, which has been previously described in patients

with CMTX,<sup>3-15</sup> but obviously the patient numbers in our study and particularly the numbers with each mutation are too small to discuss either the prevalence of CNS findings with GJB1 mutations or the specificity of CNS findings with particular mutations. It is interesting that GJB1 is expressed in the CNS in oligodendrocytes and other neuronal populations.<sup>16</sup> The MRI findings in patient 5, family 3 are of interest, particularly because she is female. Although the white matter lesions are considered definitely pathological for her age, they are non-specific. Similar MRI findings have been rarely reported with GJB1 mutations<sup>17-18</sup> but the relation of these changes to GJB1 mutations has not been established. The findings in our patient are minimal and non-specific but it is of interest that the CNS manifestations in patients with GJB1 mutations are usually mild. It would be of interest to see whether these non-specific but pathological findings are found more frequently in both male and female patients with CMTX with GJB1 mutations.

In conclusion, we describe six novel GJB1 mutations in patients with CMTX. CMTX secondary to GJB1 mutations is now the second commonest cause of CMT. GJB1 should be the first gene screened for mutations in all CMT type 1 families who are negative for the chromosome 17p11.2 duplication and who have no male to male transmission. This is particularly important in families where males are more severely affected than females, especially if the electrophysiological data suggest that males have MCVs in the demyelinating or intermediate range and females have MCVs in the axonal range, and where there is associated CNS disease. Finally, it is appropriate to consider screening for GJB1 mutations in sporadic cases, where the length of the history or the presence of foot deformity raises the possibility of CMT, and especially if the chromosome 17p11.2 duplication is negative.

#### ACKNOWLEDGEMENTS

We thank all patients participating in this study. The work was supported by the Brain Research Trust.

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Competing interests: none declared

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Received 8 January 2002  
In revised form 5 April 2002  
Accepted 22 April 2002

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*J Neurol Neurosurg Psychiatry* 2002 73: 304-306  
doi: 10.1136/jnnp.73.3.304

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