Original Article

Clinical and Genetic Features of Chinese X-linked Charcot-Marie-Tooth Type 1 Disease

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Abstract

Background: X-linked Charcot-Marie-Tooth type 1 (CMT1X) disease is one of the most common forms of inherited neuropathy caused by mutations in the gap junction beta-1 protein (*GJB1*) gene (also known as connexin 32). This study presented the clinical and genetic features of a series of Chinese patients with *GJB1* gene mutations.
Methods: A total of 22 patients from unrelated families, who were referred to Department of Neurology, Peking University First Hospital from January 2005 to January 2016, were identified with *GJB1* mutations. Their clinical records and laboratory findings were retrospectively collected and reviewed. Mutations in the *GJB1* gene were analyzed by targeted next-generation sequencing (NGS). Nucleotide alternations were confirmed with Sanger sequencing.
Results: The CMT1X patients predominantly showed distal muscle weakness of lower limbs with mild sensory disturbance. The mean age of onset was 15.6 ± 8.7 years (ranging from 1 year to 42 years). The sudden onset of cerebral symptoms appeared in four patients (18.2%); two were initial symptoms. One case had constant central nervous system (CNS) signs. There were 19 different heterozygous mutations, including 15 known mutations and four novel mutations (c.115G>T, c.380T>A, c.263C>A, and c.818_819insGGGCT). Among the 22 Chinese patients with CMT1X, the frequency of the *GJB1* mutation was 4.5% in transmembrane domain 1 (TM1), 4.5% in TM2, 22.7% in TM3, 9.1% in TM4, 4.5% in extracellular 1 (EC1), 27.3% in EC2, 9.1% in intracellular loop, 13.6% in the N-terminal domain, and 4.5% in the C-terminal domain. CMT1X with CNS impairment appeared in five (22.7%) of these patients.
Conclusions: This study indicated that CNS impairment was not rare in Chinese CMT1X patients. Mutations in the EC2 domain of the *GJB1* gene were hotspot in Chinese CMT1X patients.

Key words: Connexin 32; Gap Junction Beta-1 Protein; Neuropathy; X-linked Charcot-Marie-Tooth Type 1

Introduction

X-linked Charcot-Marie-Tooth type 1 (CMT1X) disease is a hereditary chronic progressive disease, which is caused by mutations in the gap junction beta-1 protein (*GJB1*) gene encoding the gap junction protein connexin 32 (Cx32). Clinically, the disease is characterized by the chronic progressive wasting and weakness of distal muscles in the lower limbs with sensory disturbance usually from the second decade. Some patients experience sensorineural hearing loss and central nervous system (CNS) involvement.[1-4] Nerve conduction velocity test shows a moderate neuropathy, and sural biopsy usually shows axonal or mixed axonal-demyelinating neuropathy pathology.[5,6] CMT1X has been described in Chinese patients since 2001.[3,7-19] Most of these were case reports,[8,14,17,19,20] summarized electrophysiological findings,[2,10,12-14,18] or pathological features in a few cases.[18,19]

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.204925

Here, we reported the clinical features and genetic mutations of *GJB1* gene in a large Chinese patient cohort.

Methods

Ethical approval

This study was conducted in accordance with the *Declaration of Helsinki* and was approved by the local Ethics Committee of Peking University First Hospital. Informed written consent was obtained from all patients prior to their enrollment in this study.

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Received: 15-11-2016 **Edited by:** Xin Chen
How to cite this article: Lu YY, Lyu H, Jin SQ, Zuo YH, Liu J, Wang ZX, Zhang W, Yuan Y. Clinical and Genetic Features of Chinese X-linked Charcot-Marie-Tooth Type 1 Disease. Chin Med J 2017;130:1049-54.

Patients

Ninety-two patients with CMT from unrelated families were diagnosed by next-generation sequencing (NGS) at the Department of Neurology, Peking University First Hospital from January 2005 to January 2016. Among them, a total of 22 patients with *GJB1* mutations were recruited in this study, which comprised 23.9% of the total CMT patients. As for the other CMT genes, the mutation frequency was 35.9% in peripheral myelin protein 22 gene, 17.4% in mitofusin 2 gene, 2.2% in myelin protein zero gene, 5.4% in inverted formin, FH2 and WH2 domain containing (*INF2*) gene, 4.3% in ganglioside-induced differentiation associated protein 1 gene, 2.2% in neurofilament light gene, 2.2% in periaxin gene, 1.1% in SH3 domain and tetratricopeptide repeats 2 gene, 1.1% in early growth response 2 gene, 1.1% in glycyl-tRNA synthetase gene, 1.1% in leucine rich repeat and sterile alpha motif containing 1 gene, 1.1% in FIG4 phosphoinositide 5-phosphatase (*FIG4*) gene, 1.1% in alanyl-tRNA synthetase gene, and 1.1% in dehydrogenase E1 and transketolase domain containing 1 gene.

The clinical records and electrophysiological characteristics of these 22 CMT1X patients with *GJB1* mutations were retrospectively collected and reviewed. Muscle weakness was evaluated using the Medical Research Council score. All patients were interviewed and examined by two neurologists.

Mutation analysis

Genomic DNA was extracted from the peripheral blood samples of all patients. Mutations in the *GJB1* gene were analyzed by targeted NGS. NGS panel covered all of the exons and their flanking sequences of genes known to be associated with hereditary neuropathies (gene list available on request). The exons and their flanking splice sites were captured and subsequently sequenced on an Illumina HiSeq 2500 Sequencer (Illumina, San Diego, CA, USA). The sequencing files were mapped to reference sequences with Burrows-Wheeler Aligner and Picard tools and then called with control samples with the GATK 3.0 HaplotypeCaller (Broad Institute, USA). Nucleotide alternations were confirmed with Sanger sequencing. The segregation analyses of the mutations were confirmed in the parents and the affected family members. For the novel mutations, 1000 healthy controls of Chinese origin were screened. The biological relevance of the novel amino acid changes was studied using both PolyPhen-2 (http://www.genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org/) programs.

Results

Patients' clinical and electrophysiological characteristics

These 22 patients came from unrelated families, including 20 males and 2 females. The clinical and electrophysiological characteristics of these CMT1X patients are shown in Table 1. The mean age of onset was 15.6 ± 8.7 years (ranging from 1 year to 42 years). Fifteen (68.2%) patients exhibited first symptoms in their second decade. Five (22.7%) of them presented symptoms after 20 years old. Two patients (9.1%) had an earlier onset before 10 years old, including one from infancy. The mean duration from onset to diagnostic time was 8.2 ± 4.5 years (ranging from 2 years to 18 years).

All patients showed distal muscle wasting and weakness of the lower limbs, with the involvement of the upper limbs in 13 cases. Muscle strength ranged from III to V in distal upper limbs and from 0 to V in distal lower limbs. Sensory loss in distal limbs appeared in eight cases. Pes cavus occurred in 16 cases. CNS involvement appeared in five patients (22.7%), including four cases with transient CNS symptoms and one case with constant CNS signs. Four cases (18.2%) with transient symptoms presented with sudden onset of cerebral symptoms including aphasia, dysphagia, quadriplegia, or paralysis induced by fever or infection. The symptoms usually lasted from hours to days, and the patients completely recovered without special treatment. CNS symptoms in two patients appeared initially before the development of peripheral neuropathy. One case with constant CNS involvement additionally presented with nystagmus and ataxia on physical examination.

In median nerves, the mean motor nerve conduction velocity (MCV) was 35.0 ± 4.8 m/s (range: 27.3–43.9 m/s), the mean amplitude of compound muscle action potential (CAMP) was 2.3 ± 1.5 mV (range: 0.2–5.2 mV), the mean sensory nerve conduction velocity (SCV) was 42.0 ± 9.6 m/s (range: 32.1–66.0 m/s), and the mean sensory nerve action potential (SNAP) amplitude was 5.0 ± 4.2 mV (range: 1.4–12.1 mV). In ulnar nerves, the mean MCV was 33.6 ± 5.7 m/s (range: 27.7–46.7 m/s), the mean CAMP amplitude was 3.7 ± 3.2 mV (range: 0.3–10.3 mV), the mean SCV was 40.4 ± 12.8 m/s (range: 30.3–71.0 m/s), and the SNAP mean amplitude was 3.3 ± 3.3 mV (ranging from −1.47 mV to 9.6 mV). In tibial nerves, the mean MCV was 32.5 ± 5.5 m/s (range: 23.0–39.2 m/s) and the mean CAMP amplitude was 1.1 ± 1.7 mV (range: 0.0–4.8 mV).

The cranial magnetic resonance imagings (MRIs) of patient 2 revealed abnormal signals in the splenium and genu of the corpus callosum in the first episode and in the bilateral posterior limbs of the internal capsule and splenium of the corpus callosum in the second episode. The cranial MRIs of patient 3 revealed abnormal signals in the centrum semiovale bilaterally in the first episode, the signals enlarged at the same location in the second episode, and widened at the third episode; the abnormal signals were resolved at intervals. The cranial MRIs of patient 4 revealed abnormal signals in the posterior limb of the internal capsule and the periventricular area bilaterally. The MRIs of patient 13 revealed abnormal signals in the bilateral centrum semiovale and splenium of the corpus callosum [Figure 1].

Gap junction beta-1 protein mutations

This study identified 19 different heterozygous mutations in these 22 CMT1X patients [Table 2]. The c.44G>T, c.59T>G, c.62G>A, c.194A>G, c.379A>T, C.403_404insT, c.424C>T, c.425G>A, c.490C>T, c.533A>G, c.548G>A, c.547C>T, c.556G>A, c.590C>T, and c.614A>G mutations

Table 1: The clinical and electrophysiological characteristics of 22 Chinese CMT1X patients in this study

Patient number	Age (years)	Gender	Onset age (years)	Muscle strength in distal UL	Muscle strength in distal LL	Sensory loss	CNS lesions	CMAP in median nerves (mV)	MCV in median nerves (m/s)	SNAP in median nerves (mV)
1	34	Male	21	IV	II	−	−	2.7	35.5	NE
2	14	Male	11	IV	IV	−	+	2.5	36.0	12.1
3	23	Male	8	V^{-}	I–II	−	+	NE	NE	NE
4	13	Male	10	III	I–II	−	+	0.8	28.9	NE
5	20	Female	15	IV	0–II	−	−	2.1	38.0	NA
6	24	Male	14	V	V^{-}	−	−	2.7	43.9	8.7
7	17	Male	14	V	IV	−	−	5.2	34.5	3.6
8	18	Female	12	V^{-}	IV	−	−	3.0	35.0	1.87
9	37	Male	24	V^{-}	I–II	−	+	0.3	27.3	2.3
10	19	Male	10	V^{-}	IV	UL, LL	−	NA	NA	NA
11	50	Male	32	IV	II	UL, LL	−	2.3	38.9	NE
12	34	Male	24	V^{-}	IV^{-}	LL	−	NA	NA	NA
13	15	Male	10	V	0–V^{-}	−	+	NA	NA	NA
14	24	Male	14	V	0–V^{-}	LL	−	4.7	38.0	8.7
15	13	Male	11	V	IV	UL, LL	−	NA	28.0	NA
16	44	Male	42	V	IV	−	−	NA	NA	NA
17	26	Male	18	V	I	LL	−	3.4	39.8	NE
18	27	Male	15	IV	II	UL, LL	−	0.2	NA	1.4
19	19	Male	12	IV	I–II	UL, LL	−	0.8	28.9	NE
20	21	Male	11	V	I–IV	−	−	1.8	33.9	1.4
21	19	Male	14	V	0–IV	−	−	NA	NA	NA
22	12	Male	1	IV	IV	−	−	NA	37.9	NA

Patient number	SCV in median nerves (m/s)	CMAP in ulnar nerves (mV)	MCV in ulnar nerves (m/s)	SNAP in ulnar nerves (mV)	SCV in ulnar nerves (m/s)	CMAP in tibial nerves (mV)	MCV in tibial nerves (m/s)
1	NE	NA	NA	NA	NA	NA	NA
2	41.0	1.8	31	1.8	34.0	NA	NA
3	NE	4.7	33.1	NE	NE	NE	NE
4	NE	10.3	27.7	NE	NE	3.5	38.4
5	NA	0.7	46.7	NA	NA	0.3	38.4
6	43.0	4.8	33.1	4.6	37.6	1.0	NA
7	40.6	NA	NA	2.9	35.3	0.2	32.4
8	38.6	4.4	32	−1.47	35.8	0.1	27.7
9	32.1	1.9	30.9	2.0	30.3	0.1	29.2
10	NA	NA	NA	NA	NA	NA	NA
11	NE	NA	NA	NE	NE	NE	NE
12	NA	NA	NA	NA	NA	NA	NA
13	NA	NA	NA	NA	NA	NA	NA
14	66.0	1.5	32	9.6	71.0	0.6	32.0
15	NA	NA	NA	NA	NA	4.8	32.4
16	NA	NA	NA	NA	NA	NA	NA
17	NE	3.2	41.7	5.7	37.6	NE	NE
18	42.0	0.3	NA	1.4	41.7	0.2	NA
19	NE	9.1	27.7	NE	NE	NA	NA
20	36.4	1.8	34	NE	NE	0.0	23.0
21	NA	NA	NA	NA	NA	NA	NA
22	38.2	NA	NA	NA	NA	NA	39.2

CMT1X: X-linked Charcot-Marie-Tooth type 1; LL: Low limbs; UL: Upper limbs; +: Positive; −: Negative; CNS: Central nervous system; MCV: Motor conduction velocity; CMAP: Compound motor action potential; SCV: Sensory conduction velocity; SNAP, Sensory nerve action potential; NE: Not elicited; NA: Not available.

were reported previously. Four (c.115G>T, c.263C>A, c.380T>A, and c.818_819insGGGCT) were novel mutations. Patient 22 with a c.818_819insGGGCT mutation had an X-linked family history. Five family members had peripheral neuropathy. Patients with c.115G>T, c.380T>A, and c.263C>A mutations were sporadic, and the mutation was not found in their parents. All novel mutations were not found in 1000 healthy controls and also not found in

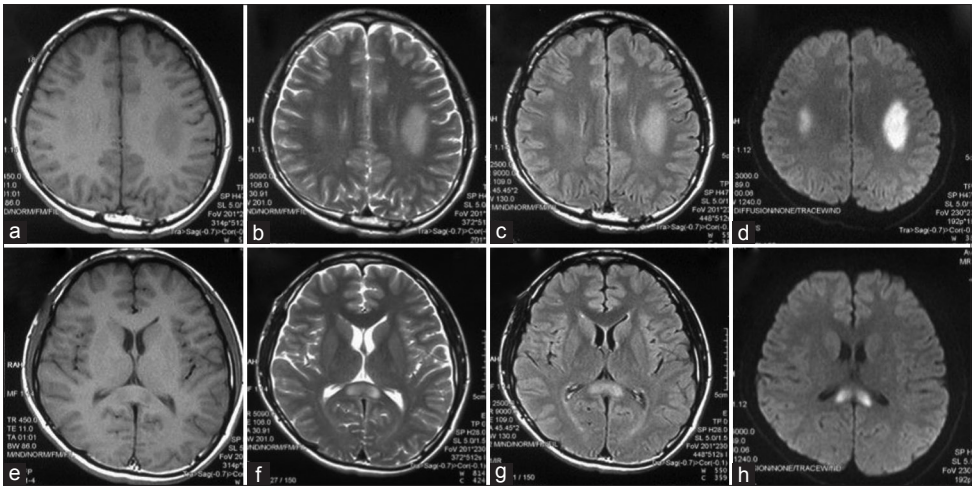

Figure 1: Brain magnetic resonance imagings of a male patient with X-linked Charcot-Marie-Tooth type 1 (patient 13) revealed abnormal signals in the bilateral centrum semiovale (a–d) and splenium of the corpus callosum (e–h). (a and e): T1 weighted; (b and f): T2 weighted; (c and d): Fluid attenuated inversion recovery; and (d and h): Diffusion-weighted magnetic resonance imaging.

Table 2: *GJB1* mutations in 22 CMT1X patients from unrelated families in this study

Patient number	Nucleotide changes	Amino acid changes	Domain	Novel mutation
1	c.44G>T	R15L	N-terminal domain	No
2	c.59T>G	I20T	N-terminal domain	No
3	c.62G>A	G21D	N-terminal domain	No
4	c.115G>T	A39S	TM1	Yes
5	c.194A>G	Y65C	EC1	No
6	c.263C>A	A88D	TM2	Yes
7	c.379A>T	I127F	IC	Yes
8	c.380T>A	I127N	IC	No
9	C.403_404insT	Y135fsX146	TM3	No
10	c.424C>T	R142W	TM3	No
11	c.424C>T	R142W	TM3	No
12	c.425G>A	R142Q	TM3	No
13	c.425G>A	R142Q	TM3	No
14	c.490C>T	R164W	EC2	No
15	c.533A>G	D178G	EC2	No
16	c.548G>A	R183H	EC2	No
17	c.548G>A	R183H	EC2	No
18	c.547C>T	R183C	EC2	No
19	c.556G>A	E186K	EC2	No
20	c.590C>T	A197V	TM4	No
21	c.614A>G	N205S	TM4	No
22	c.818_819insGGGCT	L273fs	C-terminal domain	Yes

CMT1X: X-linked Charcot-Marie-Tooth type 1; EC: Extracellular domain; TM: Transmembrane domain; IC: Intracellular loop; *GJB1*: Gap junction beta-1 protein.

NCBI SNP database, indicating that they were not benign polymorphisms. The novel missense mutations (c.115G>T, c.380T>A, and c.263C>A) were highly conserved in the Cx32 proteins across all mammalian species. The pathogenicities of the novel missense mutations were predicted to be possibly damaging (c.380T>A) and probably damaging (c.115G>T and c.263C>A) by Polyphen-2 and disease causing by Mutation Taster software. Patient 12 with pure peripheral neuropathy and patient 13 with CNS impairment had the same c.425G>A mutations.

Among 22 patients, the frequency of the *GJB1* mutations was 4.5% in transmembrane domain 1 (TM1), 4.5% in TM2, 22.7% in TM3, 9.1% in TM4, 4.5% in extracellular 1 (EC1), 27.3% in EC2, 9.1% in intracellular loop, 13.6% in the N-terminal domain, and 4.5% in the C-terminal domain [Figure 2].

Discussion

This study confirmed that CMT1X was a common form of inherited neuropathy in Chinese patients with CMT

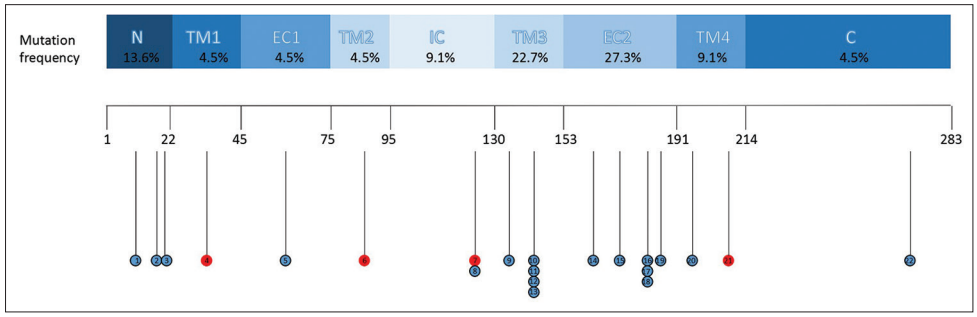

Figure 2: Distribution of the respective amino acid changes on the connexin 32 protein structure identified in 22 patients with X-linked Charcot-Marie-Tooth type 1. Different domains are indicated by rectangles with different colors. The positions of novel mutations are indicated in red.

because 23.9% of all 92 CMT families were CMT1X. This study observed that the proportion of CMT1X in Chinese population was greater than other reports. The proportion of CMT1X patients for all CMTs was 10.7% in Europe ($n = 997$),[21] 15.2% in the USA ($n = 527$),[22] 12.0% in Australia ($n = 224$),[23] and 10.9% in Japan ($n = 128$).[24] In a study of multi-ethnic Malaysian patients with CMTs ($n = 25$), CMT1X patients were all Chinese and accounted for 24% of the total CMT patients.[25]

In this study, all CMT1X patients showed distal muscle weakness predominantly in the lower limbs with a mean onset age of 15.6 ± 8.7 years, similar to other reports.[22] Most patients began presenting their symptoms in their second or third decade. Patients with an earlier onset before 10 years old or in infancy were rare in the present study and other reports.[22,26,27] Electrophysiological findings of our patients further confirmed that CMT1X is an intermediate neuropathy.

Several types of hereditary neuropathies were associated with other organ involvement, such as distal motor neuropathy with optic atrophy,[28] hereditary transthyretin amyloidosis with cardiomyopathy,[29] and dominant inherited intermediate CMT with focal segmental glomerulosclerosis.[30] This study confirmed that CNS involvement was common in Chinese CMT1X. Dysarthria and hemiparesis were the main symptoms of our patients, and other similar symptoms have been occasionally reported in other countries.[31-36] CNS symptoms developed after peripheral neuropathy symptoms in some patients in the present study, which has also been reported in previous studies.[32,36] Brain MRIs showed abnormal transient lesions involving bilateral white matter in all patients. However, the involvement of the corpus callosum which was frequently reported did not appear in all cases in the present study.[34,37] The isolated involvement of the centrum semiovale, internal capsule, and the periventricular area appeared in the present study as well as in other reports.[38,39]

We found that the EC2 domain of Cx32 protein was more affected in our patients. The EC2 domain was a hotspot mutation domain and was affected in 44% of Korean patients,[40] which was more frequent compared to our patients. Mutations in the EC1 and EC2 domains of Cx32 were in 65% of the patients with Spanish or Portuguese descent.[41] However, EC1 and EC2 were not hotspot mutation domains in Japanese[24] and Malaysian[25] patients. We found no relationship between the position of mutations and CNS involvement in CMT1X. In the present series, mutations of five patients with CNS involvement were located in the N-terminal, TM1, and TM3 domains of *GJB1* gene. The c.425G>A mutation was found in patients with pure peripheral neuropathy as well as in patients with additional CNS involvement. Four novel mutations in *GJB1* gene were found in our cohort, which expands the spectrum of mutations in CMT1X.

In summary, CNS impairment was not rare in Chinese CMT1X patients. The CMT1X diagnosis should be considered in children with transient CNS impairment with or without any signs of peripheral neuropathy. Mutations in the EC2 domain of the *GJB1* gene were hotspot in Chinese CMT1X patients.

Acknowledgments

We would like to thank the participating patients and their families for their cooperation. We also thank Ms. Jing Liu, Ms. Yue-Huan Zuo and Ms. Qiu-Rong Zhang for their technical assistance in the preparation of nerve biopsies.

Financial support and sponsorship

This study was supported by a grant from the National Science Foundation of China (No. 81471185).

Conflicts of interest

There are no conflicts of interest.

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