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Case Report

Three novel mutations in a group of Chinese patients with X-linked Charcot-Marie-Tooth disease

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ABSTRACT

The X-linked form of Charcot-Marie-Tooth disease type1 (CMTX1) is the second most common hereditary motor and sensory neuropathy caused by mutations in the gap junction beta 1 (GJB1) gene. Here, we report the clinical and genetic features of six unrelated Chinese patients with CMTX1, which were identified by genetic analysis. Among the 6 identified mutations, 3 were previously unknown (c.31A > T, c.42 C > G and c.423 del C). The six patients showed typical signs of CMT with a median age of onset of 16.5 years (range: 13–30). Sensorineural hearing loss was confirmed in the patient with the c.423 del C mutation. White matter lesions on brain magnetic resonance imaging (MRI) were observed in two patients. The three newly identified GJB1 mutations expand the clinical and mutational spectrum of CMTX1.

1. Introduction

The X-linked form of Charcot-Marie-Tooth disease type1 (CMTX1), which is caused by mutations in the gap junction beta 1 (*GJB1*) gene, is the second most common subtype of this disease [1]. *GJB1* encodes connexin32 (Cx32), which takes part in the formation of gap junctions that shorten the diffusion pathway of ions and small molecules between cells. More than 400 *GJB1* mutations have been identified to date, affecting all domains of Cx32 [1]. Here, we report the clinical features and mutation analysis of six unrelated Chinese families with CMTX1 identified in a single medical center over a period of 5 years.

2. Case report

2.1. Clinical features

Six patients with *GJB1* mutations among seventy-six patients with suspected CMT, were diagnosed by targeted next-generation sequencing (NGS), and were recruited for this study at the Beijing Tiantan Hospital from January 2012 to January 2017. All the patients were interviewed and examined by at least two neurologists.

The six patients, five males and one female (patient 6), came from six unrelated families. All patients had a positive family history for the disease. The five male CMTX1 patients presented their first symptoms in the second decade of life, while the female patient complained about tripping over at 30 years of age. None of the patients had disease onset before the age of 10 years. Unsteady gait was the first symptom in this group. All except patient 1 had independent walking ability at the time of diagnosis. None complained of decreased sensation in distal limbs. Neurological examinations showed that the patients' muscle strength ranged from 4 to 5 in the distal upper limbs and from 1 to 4 in the distal lower limbs. Pes cavus was present in all patients to varying degrees. Lamellar hyperintense signal on T2 and diffusion-weighted (DWI) magnetic resonance imaging (MRI) sequences of corpus callosum and bilateral centrum semiovale was found in two patients (patients 2 and 5). Mild hearing loss in the high frequency range was found in patient 1. Serum creatine kinase activities were normal in all except patient 3, who had mildly elevated levels. The clinical characteristics of the six CMTX1 patients are shown in Table 1.

2.2. Electrophysiological features

Electrophysiological studies were carried out using standard techniques. Motor and sensory nerve conduction was examined in the median, ulnar, peroneal, and tibial nerves. All results of nerve conduction tests were assessed against the reference values established in our laboratory, which correspond closely to values reported in the United States [2].

Electrophysiological results from all six patients demonstrated abnormal nerve conduction. Generally, the electrophysiological data suggested a sensorimotor polyneuropathy with absent sensory nerve action potentials in lower limbs in all patients. All but one patient had

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Table 1
Clinical and genetic features of the patients with *GJB1* mutations.

Patients	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Exam age/Onset	21/15	26/20	33/13	31/18	15/13	35/30
Gender	Male	Male	Male	Male	Male	Female
Muscle weakness	DLL	DLL	DLL > DUL > PLL	DLL > DUL > PLL	DLL > DUL	DLL > DUL
Muscle atrophy	Yes	Yes	Yes	Yes	Yes	Yes
Claw hand	Yes	No	Yes	Yes	Yes	No
Pes Cavus	Yes	Yes	Yes	Yes	Yes	Yes
Tendon reflexes	Decreased	Decreased	Absent	Absent	Absent	Absent
Sensory loss	Yes	Yes	Yes	Yes	Absent	Yes
Median motor CMAP/MCV(mVm/s)	9/31	3/39	1/29	4/37	5/35	2/34
Nucleotides	c.423 del C	c.425 G > A	c.42 C > G	c.548 G > A	c.103 G > A	c.31A > T
Amino acids	F141fs	R142Q	N14K	R183H	V35M	S11C

Abbreviations: DLL, distal lower limbs; DUL, distal upper limbs; PLL, proximal lower limbs; PUL, proximal upper limbs; MCV, motor nerve conduction velocity; CMAP, compound muscle action potential.

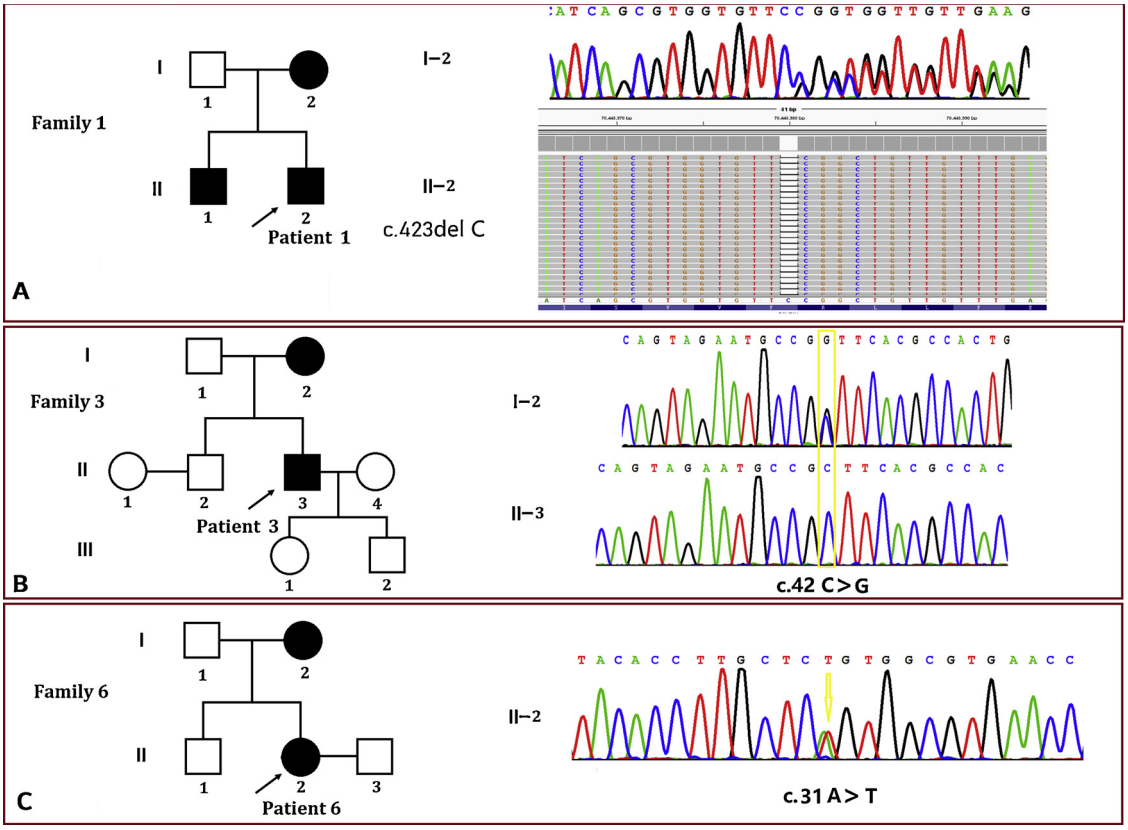

Fig. 1. Sequences of 3 novel mutations of *GJB1*.
(A) The c.423 del C mutation of *GJB1* was identified in patients from family 1.
(B)The c.42 C > G mutation of *GJB1* (square frame) was identified in patients from family 3.
(C)The c.31A > T of G*JB1* mutation (arrow) was identified in the patient from family 6.

median nerve motor conduction velocity < 38 m/s (Table 1).

2.3. Molecular analysis

Genomic DNA was extracted from peripheral venous blood samples of all probands and some affected family members after informed consent was obtained as per the study protocol approved by the Beijing Tiantan Hospital Ethics Committee. Targeted NGS was performed according to standard procedures. The NGS panel covered all exons and flanking sequences of 99 genes known to be associated with CMT, including PMP22, myelin protein zero (*MPZ*), *GJB1*, *LITAF*, etc. [3]. The suspected candidate disease-causing mutations were further verified by Sanger sequencing. Segregation analyses of the mutations was conducted in the patients and the affected family members. For the novel mutations, data from 2000 healthy controls of Chinese origin, the database of Genomic Variants included in the 1000 Genomes Project (http://browser.1000genomes.org/index.html) and the single nucleotide polymorphism database (dbSNP) (http://www.ncbi.nlm.nih.gov/projects/SNP/) were screened. The biological relevance of the novel amino acid changes was studied using PolyPhen-2 (http://www.genetics.bwh.harvard.edu/pph2/).

NGS analysis of the six patients revealed six mutations in *GJB1* (Table 1). These included the five missense mutations c.31A > T(p.S11C), c.42 C > G(p.N14 K), c.103 G > A(p.V35 M), c.425 G > A(p.R142Q), and c.548 G > A(p.R183 H), as well as the deletion mutation c.423 del C (p.F141fs). Among these mutations, c.103 G > A,

c.425 G > A and c.548 G > A have previously been recognized to be pathogenic for CMTX1, while the c.31A > T, c.42 C > G and c.423 del C mutations appear to be novel (Fig. 1). None of the novel mutations were found among 2000 healthy Chinese controls, the public database of the 1000 Genomes Project, or in the NCBI SNP database. Polyphen2 predicted *GJB1* c.31A > T (p.S11C) to be probably damaging with a score of 0.996 (sensitivity: 0.55, specificity: 0.98), and *GJB1* c.42 C > G (p.N14 K) to be probably damaging with a score of 0.998 (sensitivity: 0.27, specificity: 0.99).

3. Discussion

In this study, we found six disparate *GJB1* mutations in six unrelated patients with typical clinical presentation. Similar to other reports males tend to be more severely affected than females either in onset age or in clinical features. The patient with a c.423delC mutation had difficulty in walking independently at 21 years of age. He also had sensorineural hearing loss, which was confirmed by electrophysiological investigations, while his mother with same mutation was asymptomatic. CNS involvement with transient white matter lesions has previously been observed in two CMTX1 patients, but the exact mechanism underlying this finding remains unclear [4].

We identified three novel *GJB1* mutations in this study, which were confirmed by mutation analysis, database searches and pedigree analysis. The sites affected by the novel missense mutations (c.31A > T, c.42 C > G) are highly preserved across the Cx32 proteins from all mammalian species. A definite phenotype-genotype correlation has yet to be established for CMTX1, and the deletion mutation c.423del C did not present a more severe phenotype than the other missense mutations in this study.

Hotspot mutation domains of Cx32 protein may differ between countries or ethnic groups. In Chinese CMTX1 patients, there appears to be a mutations hotspot in the extracellular 2 (EC2) domain of Cx32. [5]. Two of our novel mutations were located in the N-terminal domain of the Cx32 protein, while the third novel mutation c.423del C was located in transmembrane domain 3 (TM3).

4. Conclusion

In conclusion, we described three novel *GJB1* mutations in patients with CMTX1. The clinical phenotype of the c.423 del C mutation identified found in this study included sensorineural hearing loss in addition to classical peripheral neuropathy. Thus, the report presents an expansion of the clinical and mutational spectrum of CMTX1.

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