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# Mutation frequency for Charcot-Marie-Tooth disease type 1 in the Chinese population is similar to that in the global ethnic patients

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**Purpose:** To investigate the genetic loci/mutations among the Chinese Charcot-Marie-Tooth disease type 1 (CMT1), which accounts for approximately 70% of Charcot-Marie-Tooth; and to study the genetic heterogeneity and mutation frequency. **Methods:** CMT1A duplication and mutations at loci of *MPZ*, *Cx32/GJB1*, *EGR2*, and *LITAF/ SIMPLE* were analyzed among 32 clinically diagnosed CMT1 patients of Chinese ancestry. **Results:** The CMT1A duplication was detected in 62.5% (20/32) CMT1 patients. This duplication accounts for the major mutation for Chinese CMT1. Among 12 cases that have no CMT1A duplication detected, three point mutations including one (3.1%) in *MPZ* and two (6.3%) in *Cx32* were identified. No mutation was detected in genes *PMP22*, *EGR2* and *LITAF* among the remaining nine (28.1%) CMT1 patients. **Conclusion:** The mutation frequency for the Chinese CMT1 is similar to that seen in the global ethnic population. Molecular testing of the CMT1A duplication, along with the loci of *MPZ* and *Cx32,* may detect the majority of Chinese CMT1 patients. ***Genet Med* 2006:8(8):532–535.**

***Key Words:*** *Chinese population, Charcot-Marie-Tooth type 1 (CMT1), Mutation screening, Mutation frequency, Genetic heterogeneity*

Charcot-Marie-Tooth disease type 1 (CMT1) is a peripheral neuropathy characterized by distal muscle weakness and atrophy, reduced nerve conduction velocities (NCV), and demyelination and re-myelination with onion bulb formation on sural nerve biopsy. Genetically, CMT1 is heterogeneous. Many genes have been identified to underlie CMT1.[1] A 1.5-Mb tandem duplication in band 17p11.2 which comprises the gene of peripheral myelin protein 22 (*PMP22*) (MIM# 601097) is the most frequent cause of CMT1.[2,3] In patients who have no *PMP22* duplication detected, mutations have been found in the following genes: *PMP22*, myelin protein zero (*MPZ/P0*) in 1q23 (MIM# 159440), connexin 32 (*Cx32/GJB1*) in Xq13.1 (MIM# 304040), early growth response 2 (*EGR2/Krox-20*) in 19q21.1–q22.1 (MIM# 129010), and lipopolysaccharide-induced transcription factor (*LITAF/ SIMPLE*) in 16p13.3–p12 (MIM# 603795).[4–8]

Molecular genetic studies of CMT have been performed widely in variant genetic ethnic background populations,[9] but relatively limited in the Chinese. For analyzing the distribution of the mutations that result in CMT1 among Chinese CMT patients, we have examined the CMT1A duplication, mutations in genes *PMP22*, *MPZ*, *CX32*, *EGR2* and *LITAF* from 32 unrelated patients of Chinese ancestry who were clinically diagnosed as having CMT1.

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## MATERIALS AND METHODS

### Patient samples

A total of 32 unrelated Chinese patients, who were clinically diagnosed with CMT1 on a basis of electrophysiological criteria, have been studied. A group of 50 healthy controls were recruited after careful clinical and electrophysiological examinations. The ethnic background of patient and control groups is Han majority of Chinese population. Both groups were collected from Beijing area. Informed consent, according to the protocol approved by the Institutional Ethics Committee, was obtained from all participants.

### Genotyping of CMT1A duplication

Genomic DNA from venous blood samples was extracted with standard protocol. Genotyping of the CMT1A duplication was carried out using three microsatellite markers (D17S4A, D17S9A, and D17S9B) as previously reported.[10]

### Mutation screening

For cases that have no CMT1A duplication detected, each patient was studied for the presence of published mutations within the coding regions of *PMP22, MPZ, GJB1, EGR2* and *LITAF* genes. Six coding exons of *MPZ* were individually amplified by

the polymerase chain reaction (PCR), using previously reported PCR primers and PCR conditions.[11] The coding region of *Cx32* was amplified with three sets of primers as described by Nelis et al.[12] Four exons of the *PMP22* gene were amplified with a protocol previously described.[7] The coding region of *EGR2* was amplified with nine sets of primers.[13] Exons 2 through 4 of the *LITAF (SIMPLE)* gene were amplified with a set of primers and PCR conditions reported by Street et al.[8]

The corresponding PCR products except exon 4 of the *LITAF* gene were analyzed by single strand conformational polymorphism (SSCP) under the following conditions: five microliters of PCR product and 5 μl SSCP loading buffer were combined; each sample was denatured at 95°C for 10 minutes and then cooled on ice immediately. Each product was analyzed under the following electrophoresis conditions: 8–10% acrylamide (3% bisacrylamide); 5% glycerol; 1 μl TBE; 4°C; 1 mA per 10 bp for 10 hours. Silver staining was then applied. PCR fragments that gave abnormal SSCP patterns were sequenced with ABI 3100 automatic sequencer (Applied Biosystems, USA). The PCR products of exon 4 of the *LITAF* gene were directly sequenced, without SSCP analysis.

## RESULTS

### Detection of CMT1A duplication

Through genotyping of three microsatellite markers (D17S4A, D17S9A, and D17S9B) that are located within the chromosome 17p11.2–p12 region, CMT1A duplication was analyzed initially. Three distinct bands or two bands with a clear dosage difference[10] were interpreted as having CMT1A duplication, which was detected in 20 CMT1 patients. The distribution of mutations among CMT1 patients in this study is summarized in Table 1.

### Mutations at the *Cx32* and *MPZ* genes were found in three CMT1 patients

Abnormal SSCP patterns were found in 3 affected patients but not in non-carriers (Fig. 1A–C). DNA sequencing analysis of the PCR product with the abnormal band in Figure 1A showed a G>A exchange at nucleotide 622 in exon 2 of *Cx32* resulting in a Glutamate (Glu) to Lysine (Lys) substitution at codon 208 (Glu208Lys) (Fig. 2A). This mutation was detected in a female patient who had family history of an X-linked dominant CMT. The second point mutation corresponding to the abnormal band in Figure 1B was a C>T exchange at nucleotide 643 in exon 2 of *Cx32*, which resulted in an Arg to Trp substitution at codon 215 (Arg215Trp). This mutation was identified from an isolated male patient (Fig. 2B). Both of the *CX32* mutations have been reported previously.[14] An intron mutation corresponding to Figure 1C, which caused a splicing error, was detected in the *MPZ* gene. It was a G>T substitution at -1 of 3′-splice site in intron 3 (c.449-1G>T) (Fig. 2C). This mutation has been reported in a Korean family[15] with autosomal dominant classical CMT1 phenotype (Table 2).

### No point mutation found among *PMP22*, *EGR2* and *LITAF* genes

Mutation screening with SSCP among genes *PMP22*, *EGR2* and *LITAF* was performed in the remaining nine patients who have no mutation detected in either CMT1A duplication or point mutation in genes *Cx32* or *MPZ*. Although we did not find any mutation in *PMP22* and *EGR2* genes, one variation c.274A>G (Ile92Val) was detected in gene *LITAF* (data not shown) among four patients who were heterozygous. This variation was reported as a polymorphism previously.[16]

**Table 1**
The distribution of mutations in CMT1 patients

| Ethnic group | *CMT1A*-duplication | Non-duplicated cases | | | | | | Total | References |
|---|---|---|---|---|---|---|---|---|---|
| | | *PMP22* | *MPZ* | *Cx32* | *EGR2* | *LITAF* | Uninformative | | |
| Asians | | | | | | | | | |
| China | 20 (62.5%) | 0 | 1 (3.1%) | 2 (6.3%) | 0 | 0 | 9 (28.1%) | 32 | This study |
| Korea | 15 (53.6%) | 1 (3.6%) | 1 (3.6%) | 2 (7.1%) | 1 (3.6%) | — | 7 (25.0%) | 28 | Choi et al.[15] |
| Japan | 40 (31.3%) | 6 (4.7%) | 12 (9.4%) | 14 (10.9%) | — | — | 56 (43.8%) | 128 | Numakara et al.[17] |
| Europeans | | | | | | | | | |
| Italy | 98 (57.6%) | 2 (1.2%) | 4 (2.4%) | 12 (7.1%) | — | — | 54 (31.8%) | 170 | Mostacciuolo et al.[18] |
| Russia | 58 (53.7%) | 2 (1.9%) | 5 (4.6%) | 8 (7.4%) | — | — | 32 (29.6%) | 108 | Mersiyanova et al.[19] |
| Europe | 579 (70.7%) | — | — | — | — | — | — | 819 | Nelis et al.[20] |
| Spain | 86 (64.7%) | — | — | — | — | — | — | 133 | Bort et al.[21] |
| USA | 79 (56.4%) | 3 (2.1%) | 5 (3.6%) | 8 (5.7%) | 1 (0.7%) | — | 44 (31.4%) | 140 | Boerkoel et al.[22] |
| | 43 (68.3%) | — | — | — | — | — | — | 63 | Wise et al.[23] |
| Global | 79 (73.8%) | 3 (2.8%) | 5 (4.7%) | 8 (7.5%) | 1 (0.9%) | — | 11 (10.3%) | 107 | Szigeti et al.[9] |

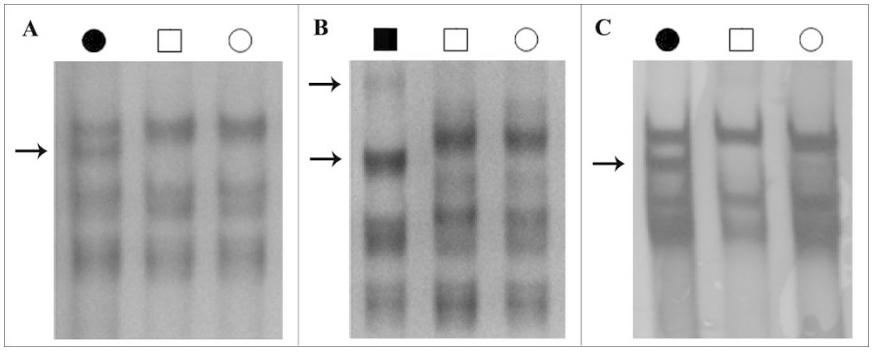


**Fig. 1.** SSCP analysis showing the altered mobility pattern of PCR fragments of the *Cx32* and *MPZ* in 3 CMT1 cases. Filled symbols represent subjects with CMT1 and open symbols represent normal subjects. Arrows indicate the abnormal bands. (A) An altered mobility shift corresponding to exon 2 of *Cx32* was found in a female patient who had a family history of an X-linked dominant CMT. (B) Altered mobility shifts corresponding to exon 2 of *Cx32* were found in an isolated male patient. (C) An altered mobility shift corresponding to exon 4 of *MPZ* was detected in an isolated female patient.

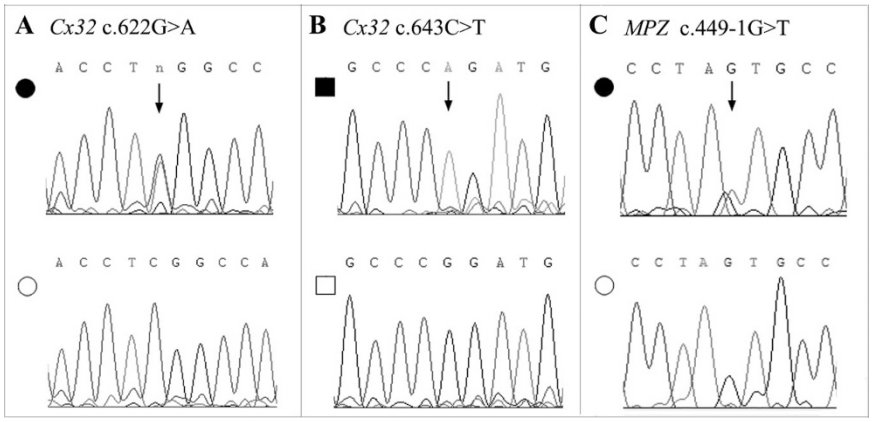


**Fig. 2.** Causative mutations in *Cx32* and *MPZ* identified by DNA sequencing. Filled symbols represent subjects with CMT1 and open symbols represent normal subjects. Arrow indicates the mutation site. (A) Corresponding to Figure 1A, a G>A exchange at nucleotide 622 in exon 2 of the *Cx32* gene resulting in a glutamate to Lysine substitution at codon 208 (Glu208Lys) was detected by sequencing the reverse strand of the PCR products. (B) Corresponding to Figure 1B, a C>T exchange at nucleotide 643 in exon 2 of the *Cx32* gene resulting in a Arg to Trp substitution at codon 215 (Arg215Trp) was detected by sequencing the reverse strand of the PCR products. (C) Corresponding to Figure 1C, a G>T exchange at -1 of 3′-splice site in intron 3 (c.449-1G>T) of the *MPZ* gene was detected by sequencing the forward strand of the PCR products.

**Table 2**
Single base pair substitutions in loci *Cx32, MPZ* and *LITAF*

| Gene-exon | Nucleotide change | Amino acid change | Inheritance | References |
|---|---|---|---|---|
| *Cx32*-Exon2 | c.622G>A | Glu208Lys | X-linked dominant | Fairweather (1994) |
| *Cx32*-Exon2 | c.643C>T | Arg215Trp | Isolated | Fairweather (1994) |
| *MPZ*-Intron3 | c.449-1G>T | 3′-splice site | Isolated | Choi (2004) |
| *LITAF*-Exon3 | c.274A>G | Ile92Val | Polymorphism | Saifi (2005) |

Nucleotide numbering: the A of ATG translation initiation site as +1.

## DISCUSSION

Molecular genetic studies of CMT have been widely performed in different ethnic background populations, but relatively limited in the Chinese. In the present study, we investigated 32 CMT1 Han-Chinese patients.

CMT type 1 (CMT1) accounts for about 70% of CMT and the majority mutation for it is associated with the CMT1A duplication. We detected the CMT1A duplication and found the duplication frequency in Chinese patients was 62.5% (Table 1). This is similar to those (53.6–70.7%) reported in global ethnic CMT1 groups,[15,18–23] but is higher than 31.2% in Japanese CMT1 patients.[17]

Twelve patients without CMT1A duplication were further screened for mutations in genes *PMP22, MPZ, Cx32, EGR2* and *LITAF*. Two mutations (Glu208Lys and Arg215Trp) in *Cx32* and one mutation (c.449-1G>T) in *MPZ* were identified. These three mutations have been reported previously,[14,15] but were not reported in the Chinese as yet. Our finding of these three mutations in the Chinese CMT1 patients indicates that there is no ethnic difference regarding the distribution of the mutation.

The mutation frequency in *Cx32* (6.3%) and *MPZ* (3.1%) evaluated in this study was analogous to those (*Cx32,* 5.7–l7.4%; *MPZ,* 2.4–4.6%) presented in several European and Asian groups (Table 1),[15,18–23] but was lower than that of the Japanese (*Cx32,* 10.9%; *MPZ,* 9.4%).[17]

In our current study, no mutation was detected in coding regions of the *PMP22, EGR2* and *LITAF* genes. Only one variation (Ile92Val) in the *LITAF* gene, which was not considered as a causative mutation but a polymorphism,[16] was detected in 4 of 12 patients in our study. The frequency of this variation is the same as that previously reported.[16] Population data showed that the mutation frequencies of the *PMP22* (1.2–4.7%) and *EGR2* (0.7–3.6%) genes were low[15–19,22] when analyzing the mutations in *LITAF* among 192 American CMT patients. Their results indicated that the mutation in *LITAF* is not a common cause of CMT.

In addition to the duplication of CMT1A and the mutations in genes *PMP22, MPZ, Cx32, EGR2* and *LITAF,* many other mutations and chromosome loci, such as *NEFL, GDAP1, MTMR2, PRX, LMNA* related to CMT, have been reported (reviewed by Zhang and Zhong).[1] The CMT cases, in which we did not find the underlying genetic defect in our current study, may be caused by the genes mentioned here, although we did screen the *LMNA* locus,[24] for which further investigation should be performed. Nevertheless, finding the CMT1A duplication, along with the mutation at genes *MPZ* and *Cx32*, accounts for the majority of Han-Chinese CMT1 patients, as seen in the global ethnic population. This made it possible to offer clinical molecular testing for the Chinese CMT1 patients.

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