Mutation Analysis of the Small Heat Shock Protein 27 Gene in Chinese Patients With Charcot-Marie-Tooth Disease

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**Background:** Charcot-Marie-Tooth (CMT) disease, the most common hereditary peripheral neuropathy, is highly clinically and genetically heterogeneous, and mutations in at least 18 genes have been identified. Recently, mutations in small heat shock protein 27 (Hsp27) were reported to cause CMT disease type 2F and distal hereditary motor neuropathy.

**Objective:** To investigate the frequency and phenotypic features of an Hsp27 mutation in Chinese patients with CMT disease.

**Design:** DNA samples from 114 unrelated patients with CMT disease were screened for mutations in Hsp27 by polymerase chain reaction and direct sequencing. A cosegregated study was performed using the MbiI restriction endonuclease, and 50 healthy control subjects were analyzed. Haplotype analysis was performed using 5 short tandem repeat markers to analyze whether the families with the same mutation probably had a common ancestor.

**Results:** One missense mutation, C379T, was detected in 4 autosomal dominant families with CMT disease type 2, and haplotype analysis indicated that the 4 families probably had a common founder. The frequency of the Hsp27 mutation is 0.9% (1/111) in Chinese patients with CMT disease in our study, and the phenotypes were characterized by later onset (age, 35-60 years) and mild sensory impairments. Electrophysiological findings showed moderately to severely slowed nerve conduction velocities in lower limb nerves but normal or mildly reduced velocities in upper limb nerves.

**Conclusions:** To our knowledge, this is the first report of an Hsp27 mutation in the People’s Republic of China. The C379T mutation in Hsp27 also causes CMT disease type 2, except for distal hereditary motor neuropathy, and the phenotypes are distinct from the family with CMT disease type 2F described previously. A mutation of Hsp27 may be uncommon in Chinese patients with CMT disease.

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C H A R C O T - M A R I E - T O O T H (CMT) disease is the most common hereditary peripheral neuropathy, with a prevalence of approximately 1 in 2500,¹ and causes progressive weakness and atrophy of the distal legs and arms, with decreased or absent tendon reflexes. According to the electrophysiological and pathological investigations, it can be further divided into 2 types: CMT disease type 1, the demyelinating form, characterized by a slow motor median nerve conduction velocity (NCV) (<38 m/s); and CMT disease type 2, the axonal form, with a normal or slightly reduced NCV (≥38 m/s).²³ Charcot-Marie-Tooth disease is highly clinically and genetically heterogeneous, and mutations have been reported in at least 18 genes (information available at: Inherited Peripheral Neuropathies Mutation Database [http://molgen-www.uia.ac.be/CMTMutations/]).

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Recently, 5 missense mutations in small heat shock protein 27 (Hsp27) have been identified to cause CMT disease type 2F and distal hereditary motor neuropathy (dHMN), most of which occur in the highly conserved Hsp20 α-crystallin domain of the protein.⁴ Small heat shock protein 27 is a member of the Hsp superfamily and is abundant in different nerve cells.⁵ Several studies showed that wild-type Hsp27 has neuroprotective action. It can protect neurons against cell apoptosis induced by kainic acid, retinoic acid, or nerve growth factor withdrawal,⁶ and its up-regulation and phosphorylation is
necessary for sensory and motor neuron survival following peripheral nerve injury.10 Reversely, mutant Hsp27 can reduce the viability of neuronal cells and impaired neurofilament assembly.4

To study the Chinese patients with CMT disease for mutations in Hsp27 and to analyze its phenotypic features, we screened 114 individuals and detected a missense mutation (C379T) in 4 autosomal dominant families with CMT disease type 2. We also reported the clinical electrophysiological features of the patients with the detected mutation.

### Methods

#### Patients

This study included 114 unrelated individuals with CMT disease. All patients were diagnosed as having the disease by 2 neurologists (B.T., X.L., G.Z., W.L., C.Z., B.C., F.Z., L.S., R.Z., or H.J.) according to the diagnosis criteria of CMT disease type 2, and they were all of Han nationality. Informed consent was obtained, and 50 healthy control subjects were screened to determine whether the detected sequence variation was a polymorphism or a mutation. Following detection of the mutation in an
A family with the C379T mutation was identified in a Chinese isolate of CMT disease. The mutation was identified in 114 unrelated patients with CMT disease, and discovered a C379T mutation that generated the amino acid alterations of Arg127Trp in 4 families with autosomal dominant CMT disease type 2 (Figure 1). These 4 families all came from the Hunan province. The mutation was heterozygous, segregated perfectly with the CMT disease phenotype, and was absent in 50 healthy controls (Figures 2, 3, 4, and 5). We also compared the disease-segregating haplotypes in relatives of the 4 families (if available). Haplotype analysis indicated that 3 families (1, 3, and 4) had a common ances-
tor and the other family (2) was also closely related to them, suggesting that the 4 families probably had a common founder.

CLINICAL INFORMATION OF PATIENTS WITH THE HSP27 MUTATION

At least 1 affected member of each family was examined. According to the diagnosis criteria,11 families 1, 2, and 4 were diagnosed as having CMT disease type 2. Family 3 cannot be divided according to present records. The clinical and electrophysiological features of the 4 families are summarized in Table 2 and Table 3 in detail. The initial symptoms in all patients were difficulty in walking, followed by weakness and atrophy of the distal parts of the limbs. Tendon reflexes were depressed or absent, with mild stocking sensory loss to pricking pain or vibration in the feet. Talipes cavus or clawhand deformity was observed in most of the patients. Other symptoms, including cramps and fasciculations in the lower limbs and numbness and tingling in the feet, were seen in 1 patient each. There was no cranial nerve involvement and no cerebellar or pyramidal signs in all patients. Patient III:2 of family 2, aged

Figure 3. Family 2 and haplotype analysis (A) and the restricted digest of the family members (B). In B, the abnormal migration alteration is indicated (arrow). An explanation of all symbols/abbreviations is given in the legend to Figure 2.

Figure 4. Family 3 and haplotype analysis (A) and the restricted digest of the family members (B). In B, the abnormal migration alteration is indicated (arrow). An explanation of all symbols/abbreviations is given in the legend to Figure 2.
37 years, patient III:3 of family 3, aged 23 years, and patient IV:2 of family 4, aged 29 years, who were mutation carriers, were asymptomatic and might be presymptomatic patients, which could be due to age-dependent penetrance. The mean±SD age of onset was 46.29±9.01 years, and the distal arms were involved a mean±SD of 13.17±6.68 years later. Patients required a walking stick after a mean±SD of 12.60±4.93 years and a wheelchair after a mean±SD of 20.67±5.69 years after the onset. The duration of the disease was a mean±SD of 17.57±6.63 years.

Figure 5. Family 4 and haplotype analysis (A) and the restricted digest of the family members (B). In B, the abnormal migration alteration is indicated (arrow). An explanation of all symbols/abbreviations is given in the legend to Figure 2.
The electrophysiological investigations showed moderately to severely diminished motor and sensory NCVs or no nerve action potentials in the lower limb nerves but normal or mildly reduced velocities and potentials in the upper limb nerves. The result of a sural nerve biopsy performed on patient II:1 of family 2 (Figure 6) was consistent with chronic axonal neuropathy.

Small heat shock proteins are a superfamily of proteins that vary from 15 to 30 kDa and share the \( \alpha \)-crystallin domain, a conserved sequence of 85 to 100 amino acid residues in the C-terminus. Ten Hsps are identified: HspB1 through HspB10. Mutations in 4 Hsps (Hsp27, Hsp22, \( \alpha \)-crystallin, and \( \beta \)-crystallin) are associated with diseases, including CMT disease type 2, dHMN, congenital cataract, myofibrillar myopathy, and desmin-related myopathy, suggesting that the Hsp family may play an important role in the pathogenesis of neurological and muscular disorders. Small heat shock proteins are part of signal transduction cascades and have molecular chaperone-like properties, and they can increase cell survival under stress conditions by inhibiting apoptosis.

In this study, we screened 114 Chinese patients with CMT disease phenotypes and identified a missense mutation (C379T) in Hsp27 that has been reported recently. This mutation was found in 4 autosomal dominant families with CMT disease type 2, and haplotype analysis indicated that the 4 families probably had a common founder. To our knowledge, this is the first report of the Hsp27 mutation in the People’s Republic of China. The frequency of the Hsp27 mutation is 0.9% (1/111) in Chinese patients with CMT disease in our study. Evgrafov et al also screened 301 unrelated individuals with CMT disease.

**Table 2. Clinical Features of the Patients With the Hsp27 Mutation**

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>Age at Onset, y</th>
<th>Duration of the Disease, y</th>
<th>Hands Involved, y</th>
<th>Requiring Walking Stick, y</th>
<th>Requiring Wheelchair, y</th>
<th>Upper Limbs</th>
<th>Lower Limbs</th>
<th>Sensory Disorders</th>
<th>Tendon Reflexes</th>
<th>Atrophy</th>
<th>Tendon Reflexes</th>
<th>Atrophy</th>
<th>Additional Symptoms</th>
</tr>
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<tbody>
<tr>
<td>1 II:3</td>
<td>35</td>
<td>54</td>
<td>29</td>
<td>24</td>
<td>20</td>
<td>27</td>
<td>↓</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>2 II:1</td>
<td>49</td>
<td>19</td>
<td>8</td>
<td>11</td>
<td>16</td>
<td>↓</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>3 II:5</td>
<td>42</td>
<td>17</td>
<td>9</td>
<td>15</td>
<td>0</td>
<td>↓</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Numbness and tingling in the feet</td>
</tr>
<tr>
<td>4 II:7</td>
<td>47</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>N</td>
<td>↓</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>III:1</td>
<td>37</td>
<td>20</td>
<td>18</td>
<td>8</td>
<td>19</td>
<td>↓</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>None</td>
</tr>
</tbody>
</table>

**Table 3. Electrophysiological Data of Patients With the Hsp27 Mutation**

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>MCV, m/s</th>
<th>SCV, m/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tibial</td>
<td>Peroneal</td>
</tr>
<tr>
<td>1</td>
<td>III:4</td>
<td>–</td>
<td>31.5</td>
</tr>
<tr>
<td>2</td>
<td>II:1</td>
<td>ND</td>
<td>26.7</td>
</tr>
<tr>
<td>4</td>
<td>II:7</td>
<td>41.6</td>
<td>44.7</td>
</tr>
<tr>
<td>III:1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**Figure 6.** The results of a sural nerve biopsy performed on patient II:1 of family 2. The transverse semithin section shows chronic atrophy, loss, and regeneration of myelinated axons (arrows) without signs of obvious demyelination and a reduction in the density of myelinated fibers (toluidine blue, original magnification ×400).
disease and confirmed one family with the Hsp27 mutation, suggesting that a mutation of the HSP27 gene may not be common.

In our study, the Hsp27 C379T mutation causes CMT disease type 2 phenotypes. However, this mutation in Hsp27 also causes dHMN, a disease of pure motor neuropathy, also known as spinal CMT disease, characterized by normal motor and sensory NCVs and degeneration of spinal cord anterior horn cells, which is similar to CMT disease clinically but without sensory abnormalities. A similar phenomenon is observed for the C404T mutation in Hsp27, which is also associated with CMT disease type 2 and dHMN, suggesting that even the same mutation in the same gene may lead to a variation in clinical phenotypes, but the mechanisms remain unclear.

Because CMT disease type 2 is clinically and genetically heterogeneous, it is important to describe the clinical features of the 4 mutation-detected families studied herein and to compare them with those of families with CMT disease type 2 described in the literature. Evgrafov et al found a C404T missense mutation in Hsp27 in a Russian family with CMT disease type 2F, which was reported previously. In this family, disease onset occurred between the ages of 15 and 25 years. Mild to moderate sensory impairments were observed in the feet and hands in all the patients. However, in our study, the 4 mutation-detected families were clinically characterized by late onset (range, 35-60 years) and mild sensory impairments in the feet. In addition, cramps and fasciculations in the lower limbs that were absent in the Russian family with CMT disease type 2F were seen in our study, suggesting clinical variation caused by different mutations in the same gene. The clinical manifestations, including variable ages at onset, weakness and/or atrophy in the distal muscles, reduced or absent tendon reflexes, no nerve enlargement, normal or slightly decreased motor NCVs in the median nerve, and chronic myelinated axonal atrophy, loss, and regeneration, of the 4 mutation-detected families were similar to those of patients clinically diagnosed as having CMT disease type 2. Cramps or fasciculations in the limbs in patients with CMT disease type 2 were also reported by other researchers.

In conclusion, our study confirmed that the Hsp27 mutation can cause a late-onset CMT disease type 2 phenotype with mild sensory disorder. And, we concluded that the frequency of the Hsp27 mutation may not be common in Chinese patients with CMT disease. Further efforts should include determining the molecular mechanism of mutant Hsp27 causing CMT disease and dHMN, which would be helpful for future therapeutic strategies in patients with hereditary peripheral neuropathy.

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References