Spinocerebellar Ataxia Type 1 in China

Molecular Analysis and Genotype-Phenotype Correlation in 5 Families

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Background: Twelve genetic types of autosomal dominant hereditary ataxia have been recently identified and the genes responsible for most of them cloned. Molecular identification of the type of ataxia is important to determine the disease prevalence and its natural history in various populations.

Objectives: To perform molecular analysis of 75 Chinese families affected with spinocerebellar ataxia (SCA) and to evaluate the spectrum of mutations in these genes and the correlation between genotypes and phenotypes in Chinese patients.

Setting: Neurogenetics Unit, China-Japan Friendship Hospital, Beijing, China.

Methods: One hundred nine patients from 75 kindreds diagnosed as having autosomal dominant SCA, 16 patients with sporadic SCA or spastic paraplegia, 280 control chromosomes of the Chinese population, and 120 control chromosomes of the Sakha population were selected for this study. We conducted detailed mutational analysis by direct sequencing of polymerase chain reaction products amplified from genomic DNA.

Results: Spinocerebellar ataxia type 1 (SCA1) was identified in 5 families with 12 studied patients. All affected family members were heterozygous for a CAG repeat expansion in the SCA1 gene containing 51 to 64 trinucleotide repeats. Normal alleles had 26 to 35 repeats. Spinocerebellar ataxia type 1 accounted for 7% of the studied Chinese families with ataxia. In addition, we determined the frequency of a single vs double CAT interruption in 120 control chromosomes of the Siberian Sakha population, which has the highest known prevalence of SCA1, and compared this with 280 control chromosomes from the Chinese populations. The results show that 64.7% of the Siberian normal alleles contain a single CAT interruption, whereas 92% of the Chinese had more than 1 interruption.

Conclusions: Spinocerebellar ataxia type 1 is responsible for 7% of affected families in the Chinese population. A correlation between the prevalence of SCA1 and the number of CAT interruptions in the trinucleotide chain suggests that a CAT-to-CAG substitution may have been the initial event contributing to the generation of expanded alleles and influencing relative prevalence of SCA1.

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The dominantly inherited spinocerebellar ataxias (SCAs) represent a group of genetically diverse neurological conditions that are characterized by progressive deterioration of balance due to degeneration of the cerebellum and its afferent and efferent pathways. Extracerebellar symptoms include nuclear or supranuclear ophthalmoparesis, slow saccades, pyramidal and extrapyramidal signs, and axonal neuropathy. Several genetically distinct types of autosomal dominant ataxia have been mapped: SCA type 1 (SCA1) to chromosome 6p, SCA2 to 12q, SCA3/Machado-Joseph disease (MJD) to 14q, SCA4 to 16q, SCA5 to 11cen, SCA6 to 19p, SCA7 to 3p, SCA8 to 10q24, SCA10 to 22q13, SCA11 to 15q14-21, SCA12 to 5q31-33. In 6 types, SCA1, SCA2, SCA3/MJD, SCA6, SCA7, and SCA12, the disease-causing gene has been cloned and the mutation identified as an expansion of a CAG trinucleotide repeat in the gene coding region. CAG repeat expansion is the mutational mechanism in a large group of neurodegenerative diseases that includes, in addition to ataxias, spinobulbar muscular atrophy, Huntington disease, dentatorubral pallidoluysian atrophy (DRPLA). Spinocerebellar ataxia type 1 is an autosomal dominant neurodegenerative disorder characterized by cerebellar ataxia, progressive motor deterioration, and loss of cerebellar Purkinje cells and brainstem neurons. This type of disease was first reported by Schut in a large US family of Russian extraction. The SCA1 locus was
PATIENTS AND METHODS

PATIENTS

One hundred nine individuals from 75 kindreds with autosomal dominant SCA, 16 patients with sporadic SCA or spastic paraplegia, 280 control chromosomes of the Chinese population, and 120 control chromosomes of the Sakha population were selected for this study. The affected families originated in Bejing, Shanghai, Shenyang, Hunan, Hubei, Jiangxi, Zhenjiang, Jiangsu, Hebei, and Inner Mongolia, representing the southern and northern parts of China. Table 1 shows the distribution of patients according to geographic origin. All subjects were examined by the same 3 neurologists (G.-X.W., Y.-X.Z., and B.-T.S.). Studies of families with SCA2 and SCA3/MJD have previously been reported.

DNA SAMPLES

Blood samples were collected from 125 individuals affected with autosomal dominant SCA and 16 patients with sporadic SCA or spastic paraplegia, 140 healthy Chinese controls, and 60 healthy Sakha controls after obtaining informed consent. High-molecular-weight genomic DNA was extracted from either peripheral leukocytes or lymphoblastoid cell lines transformed by Epstein-Barr virus following standard procedures.

IDENTIFICATION OF THE CAG REPEAT EXPANSION

The number of CAG repeat units in the SCA1 gene was determined by polyacrylamide gel electrophoresis of polymerase chain reaction (PCR) fragments produced by amplification with primer pair Rep-1 and Rep-2 as outlined by Orr et al and Goldfarb et al. SCA2 and SCA3/MJD alleles were amplified and analyzed on agarose and acrylamide gels using previously described methods. DRPLA alleles were amplified using primers and conditions described by Koide et al. SCA6, SCA7, SCA8, and SCA12 alleles were amplified using gene-specific primers. The antisense primer was fluorescently labeled. For PCR amplification, genomic DNA was denatured at 95°C for 2 minutes and the reaction carried out for 32 cycles consisting of 1-minute denaturation at 95°C, 1-minute annealing at 60°C, and 1-minute elongation at 72°C. This was followed by a final elongation at 72°C for 7 minutes, in a total volume of 20 µL containing 200 ng of genomic DNA; 20 pmol/µL of each primer; 200 mmol/L each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxythymidine triphosphate, and deoxyguanosine triphosphate; 50-mmol/L potassium chloride; 1.5-mmol/L magnesium chloride; 10-mmol/L tromethamine, pH 8.8; and 5 U of Taq polymerase. The PCR products were electrophoresed in a 5% denaturing polyacrylamide gel on an automated ABI 373A sequencer, and analysis was performed by using the GeneScan 672 program (ABI-Perkin Elmer, Foster City, Calif). With some samples, PCR was performed in a total volume of 20 µL containing 200 ng of genomic DNA; 20 µL of each primer; 200 µmol/L each of deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate, 200 µmol/L for each; 30-µmol/L deoxythymidine triphosphate; 370 KBq of [α-32P]-deoxycytidine triphosphate (370 MBq/µL); 50-mmol/L potassium chloride; 1.5-mmol/L magnesium chloride; 10-mmol/L tromethamine, pH 8.8; and 5 U of Taq polymerase. The PCR products were electrophoresed in 5% denaturing polyacrylamide gel together with PCR products from cloned templates of normal and expanded SCA1 alleles as size standards and subjected to autoradiography. A molecular weight standard was included to accurately determine the number of CAG repeats.

NUCLEOTIDE SEQUENCE ANALYSIS

The PCR products amplified from normal and SCA1 chromosomes were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. DNA was recovered from agarose gel plugs using the GeneClean kit (Bio101), subcloned into pGEM-T easy vector (Promega, Madison, Wis), and transfected into DH5α-competent cells. Insert-containing clones were sequenced using the Sequenase version 2.0 DNA sequencing kit. The numbers of CAG repeats and CAT interruptions were determined from sequence analysis.

STATISTICAL ANALYSIS

Correlation of the age of disease onset with the number of CAG repeat units was determined by Pearson correlation coefficient test. Other statistical analyses were performed using the t test or χ2 test.

RESULTS

SCA1 CHROMOSOMES AND GENOTYPE-PHENOTYPE CORRELATION

Most of our patients originated in the midnorthern, northeastern, mideastern, and midsouthern regions of China, such as Beijing, Shanghai, Shenyang, Hunan, Hubei, Jiangxi, Zhejiang, Jiangsu, Hebei, and Inner Mongolia. Table 1 shows the distribution of patients according to geographic origin. The SCA1 mutation was detected in 5 (7%) of 75 Chinese families with autosomal dominant SCA. All 12 patients with the SCA1 phenotype were het-
erozygous for alleles with CAG repeat numbers within a range of 51 to 64. The number of trinucleotide repeats in the normal alleles of the Chinese control subjects ranged from 26 to 35. As in all other disorders with CAG repeat expansion, we observed a statistically significant negative correlation between the age of disease onset and the number of trinucleotide repeats, with a Pearson correlation coefficient of \( r = -0.914 \).

Clinical features of the studied patients with SCA1 are summarized in Table 2. The mean ± SD age at last examination was 37.3 ± 4.3 years. Patients with SCA1 in our series shared classic clinical features of gait and limb ataxia, dysarthria, pyramidal tract signs (spasticity, hyperreflexia, and extensor plantar responses), and variable degree of oculomotor dysfunction, which includes 1 or more of the following: nystagmus, slow saccades, and ophthalmoparesis. Saccades were slowed in 50%. Gaze paralysis was most frequent in the vertical plane, usually upward (33%). Nystagmus on lateral gaze was rare. Motor weakness, amyotrophy, and mild sensory deficits manifested as proprioceptive loss were seen in some patients. Dementia was evident in 4 (33%) of 12 patients with SCA1. Although ataxia, dysarthria, and cranial nerve dysfunction were consistently present in every SCA1-affected individual, considerable intrafamilial variability was noted with regard to all of the other clinical features. Several of the kindreds that did not have an expanded SCA1 CAG repeat displayed the same clinical findings as were observed in SCA1 kindreds, reflecting the inherent difficulty of clinical classification. The frequencies of specific clinical signs were analyzed by the Mann-Whitney \( U \) test in an effort to determine whether any correlation existed with the number of CAG repeats, but none were found in our data set.

### COMPARISON OF THE CLINICAL PICTURE IN SCA1, SCA2, AND SCA3 AND RELATIVE FREQUENCY OF SCA1 AMONG OTHER TYPES OF ATAXIA

The combined frequency of SCA1, SCA2, and SCA3/MJD in the Chinese population was 53%. None of the 16 patients with sporadic SCA or spastic paraplegia tested positive for SCA1, SCA2, SCA3, SCA6, or SCA7, nor did any of our patients with inherited or sporadic ataxia test positive for SCA8, SCA12, or DRPLA, including a large family with myoclonus epilepsy, tremor, and ataxia (data not shown). Clinical finding in 12 patients from 5 SCA1 families were compared with 16 patients from 9 SCA2 families and 72 patients from 26 SCA3/MJD families in Table 2. Onset age was not statistically different. Hyperactive reflexes and spasticity were more frequent in SCA1, whereas hypoactive reflexes and slow saccades were more frequent in SCA2; facial myokymia and horizontal nystagmus were seen more frequently in SCA3/MJD. Of patients with SCA3/MJD, only 12 (46%) were correctly diagnosed on clinical grounds. These patients manifested with typical adult-onset (type II-III MJD) phenotype that included ophthalmoparesis with eyelid retraction, facial myokymia, ataxia, spasticity, and amyotrophy. The remaining 54% of the patients with SCA3/MJD had clinical features indistinguishable from patients with other types of ataxia, such as SCA1 or SCA2. Of SCA2 families, only 5 (36%) and of SCA1 families only 2 (40%) were diagnosed on clinical grounds alone. Molecular diagnosis was helpful in identifying or confirming the type of SCA in each of these cases.

### ASSOCIATION BETWEEN THE NUMBER OF CAT INTERRUPTIONS AND RELATIVE PREVALENCE OF SCA1

The results of sequence analysis showed that 65% of the Siberian Sakha and only 8% of Chinese normal alleles had a single CAT interruption. Based on historic and ethnographic studies, the Sakha (Iliakut) population, currently numbering 350,000, is a relatively recent arrival...
in eastern Siberia. The prevalence of SCA1 is extremely high in this small population, whereas the prevalence of SCA1 in the Chinese population is significantly lower.

**COMMENT**

In 1992, at an MJD international workshop, families with a dominantly inherited ataxia were discussed in detail, and several MJD families from China were described as well as other SCA-type families. The discussion and agreement that dominantly inherited ataxias were present in China led us to do a more detailed clinical-molecular analysis. Subsequently, we have had the opportunity to evaluate members representing 75 Chinese kindreds with dominantly inherited ataxia. The cloning of genes and identification of the causative mutations in several types of SCA have provided a powerful tool for establishing a definitive diagnosis by genetic testing. Our data indicate that 5 (7%) of the studied families with hereditary ataxia originating from various regions of China, including Beijing, Shanghai, Jiangsu, Zhejiang, Hebei, Liaoning, Hunan, Hubei, Jiangxi, and Inner Mongolia, had SCA1. This frequency is similar to the frequencies recently reported in other population groups. We found a strong inverse correlation between the age at disease onset and the CAG repeat number in the expanded alleles, similar to features previously described in Huntington disease and other studied SCAs.

We observed a wide spectrum of clinical features in patients with SCA1, significantly overlapping with SCA2 and SCA3/MJD, but there was no statistically significant correlation between the number of CAG repeat units in the SCA1 gene and clinical manifestations. Such correlations were previously reported in patients with SCA3/MJD and SCA2. This discrepancy may be due to the small size of our data set. The comparison of the clinical features of our patients with 3 reported series showed an overall clinical similarity (Table 3). Some statistical differences among series may be explained by differences in evaluation criteria and examiners. Meanwhile, the fact that low occurrence of slow saccades and sphincter disturbances and high occurrence of horizontal nystagmus, lower limb weakness, lower limb amyotrophy, and knee reflexes occurred in our patients with autosomal dominant cerebellar ataxia type 1 compared with 3 other large groups could be explained by occurrence of different mutations in our population (Table 4). The existence of different clinical subtypes within autosomal dominant cerebellar ataxia type 1 has been addressed.

Of the patients with SCA1 whom we studied, only 2 could be reliably diagnosed without genetic testing. A wide variety of phenotypes seen in SCA1 and a significant degree of clinical overlap with SCA2 and SCA3/MJD highlight the difficulty of making the diagnosis on the basis of clinical information alone. Of the patients with SCA2 we studied, only 5 (56%) and of the patients with SCA3/MJD only 12 (46%) could be reliably diagnosed without genetic testing. We found that SCA3/MJD, more often than other SCA types, manifests with specific features such as ophthalmoparesis and characteristic eyelid retrac-

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**Table 3. Comparison of Findings in Reported Series of Spinocerebellar Ataxia Type 1**

<table>
<thead>
<tr>
<th>Findings</th>
<th>Present Study</th>
<th>Sasaki et al.</th>
<th>Pareyson et al.</th>
<th>Dubourg et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected examined, No.</td>
<td>12</td>
<td>35</td>
<td>61</td>
<td>42</td>
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<tr>
<td>Onset age, mean ± SD, y</td>
<td>37.3 ± 4.3</td>
<td>36 ± 10</td>
<td>37.4 ± 3.3</td>
<td>33 ± 9</td>
</tr>
<tr>
<td>Signs at presentation, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cerebellar gait ataxia</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cerebellar limb ataxia</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Cerebellar dysarthria</td>
<td>83*</td>
<td>100</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Ankle reflexes decreased or absent</td>
<td>17</td>
<td>20</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Ankle reflexes increased</td>
<td>83*</td>
<td>43</td>
<td>71</td>
<td>31</td>
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<tr>
<td>Extensor plantar response</td>
<td>33*</td>
<td>83</td>
<td>...</td>
<td>40</td>
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<tr>
<td>Spasticity</td>
<td>67*</td>
<td>23</td>
<td>15</td>
<td>...</td>
</tr>
<tr>
<td>Slow saccades</td>
<td>17*</td>
<td>15</td>
<td>59</td>
<td>...</td>
</tr>
<tr>
<td>Limited eye movements (upward)</td>
<td>33</td>
<td>17</td>
<td>...</td>
<td>22</td>
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<tr>
<td>Dysphagia</td>
<td>67</td>
<td>54</td>
<td>76</td>
<td>51</td>
</tr>
<tr>
<td>Pyramidal signs</td>
<td>33*</td>
<td>...</td>
<td>77</td>
<td>...</td>
</tr>
<tr>
<td>Decreased vibration sense in the lower limbs</td>
<td>67*</td>
<td>40</td>
<td>...</td>
<td>51</td>
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<tr>
<td>Facial myokymia</td>
<td>8</td>
<td>11</td>
<td>19</td>
<td>...</td>
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<tr>
<td>Horizontal nystagmus</td>
<td>8*</td>
<td>37</td>
<td>37</td>
<td>...</td>
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<tr>
<td>Lower limb weakness</td>
<td>25</td>
<td>...</td>
<td>...</td>
<td>23</td>
</tr>
<tr>
<td>Lower limb amyotrophy</td>
<td>25</td>
<td>20</td>
<td>38</td>
<td>5</td>
</tr>
<tr>
<td>Dementia</td>
<td>33</td>
<td>20</td>
<td>32</td>
<td>5</td>
</tr>
</tbody>
</table>

*P < .001, Yates χ² test.
†Ellipses indicate that authors did not report these data.
tion, facial myokymia, ataxia, spasticity, and amyotrophy. Patients with SCA3/MJD who merely have ataxia with slowed saccades were clinically indistinguishable from patients with SCA1 or SCA2. The PCR-based testing makes it possible to diagnose the type of ataxia with great certainty. This underscores the importance of genetic testing for diagnostic accuracy in patients with autosomal dominant ataxia.

In the present study, we found a close association between the prevalence of SCA1 in Chinese and Sakha populations and the frequency of CAT interruptions in the SCA1 gene. A similar association between the relative prevalence of SCA1 (15%) in the North American population and the frequency of CAT interruptions (11%) was reported. If a substitution of CAT for CAG was the initial event contributing to generation of expanded alleles, the Sakha population must have been predisposed to such a transition by simply having more individuals carrying a single CAT interruption.

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