Frequency of SCA1, SCA2, SCA3/MJD, SCA6, SCA7, and DRPLA CAG Trinucleotide Repeat Expansion in Patients With Hereditary Spinocerebellar Ataxia From Chinese Kindreds

Beisha Tang, MD; Chunyu Liu, PhD; Lu Shen, MD; Heping Dai; Qian Pan; Lijuan Jing; Shan Ouyang; Jiahui Xia, PhD


Patients and Methods: Spinocerebellar ataxia type 1, SCA2, SCA3/MJD, SCA6, SCA7, and DRPLA (CAG)n mutation were detected with the polymerase chain reaction, highly denaturing polyacrylamide gel electrophoresis, and silver staining technique in 167 patients with autosomal dominant SCA from 85 Chinese families and 37 patients with sporadic SCA.

Results: Spinocerebellar ataxia type 1 (CAG)n mutation in 7 patients from 4 kindreds (4.70%) was expanded to 53 to 62 repeats. Spinocerebellar ataxia type 2 (CAG)n mutation in 12 patients from 5 kindreds (5.88%) was expanded to 42 to 47 repeats. Spinocerebellar ataxia type 3/Machado-Joseph disease (CAG)n mutation in 83 patients from 41 kindreds (48.23%) was expanded to 68 to 83 repeats. Sixty-five patients from 35 kindreds (41.19%) and 37 patients with sporadic SCA did not test positive for SCA1, SCA2, SCA3/MJD, SCA6, SCA7, or DRPLA. There was a predictable inverse relationship between the number of CAG repeats and the age at onset for SCA3/MJD and SCA2. Clinically, dementia and hyporeflexia were more frequent in patients with SCA2, while spasticity, hyperreflexia, and Babinski signs were more frequent in patients with SCA3/MJD, and those might be helpful in clinical work to primarily distinguish patients with SCA3/MJD and SCA2 from others with different types of SCA.

Conclusions: The frequency of SCA3/MJD is substantially higher than that of SCA1 and SCA2 in patients with autosomal dominant SCA from Chinese kindreds, who are non-Portuguese. Clinical expressions of the various types of SCAs overlap one another; therefore, for clinical study it is important to make a gene diagnosis and genetic classification for patients with SCA.

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SUBJECTS AND METHODS

PATIENTS AND METHODS

One hundred sixty-seven patients with autosomal dominant SCA from 85 kindreds and 37 patients with sporadic SCA were screened for the SCA1, SCA2, SCA3/MJD, SCA6, SCA7, and DRPLA mutations. The affected families originated in Zhejiang, Jiangsu, Shandong, Jiangxi, Jinlin, Hubei, Henan, Yunnan, Hubei, and Hunan, representing equally southern and northern China. All are Han.

Included among the major clinical features of affected persons were progressive gait and limb ataxia and dysarthria. The diagnosis of SCA was determined by clinical examination by an experienced neurologist (B.S., L.S., L.J., or S.O.) using established diagnostic criteria. Some persons had anticipation. According to the criteria of Hirayama et al.11 we divided our patients into 3 grades in accord with disease severity: grade 1, patient can walk independently or occasionally needs help; grade 2, patient completely needs support; and grade 3, patient is only wheelchair bound or bedridden.

Ages at onset were based on information provided by the patient, a close relative, or both. The mean (±SD) age at onset was 34.23 ± 15.72 years (age range, 12-69 years) for 167 patients with autosomal dominant SCA, and 34.10 ± 17.85 years (age range, 13-58 years) for 37 patients with sporadic SCA. The mean (±SD) duration of disease was 8.37 ± 6.31 years (duration range, 1-18 years) for 37 patients with sporadic SCA.

RESULTS

CAG EXPANSIONS

Frequency of the SCA1 Expanded Allele

One hundred sixty patients from 80 kindreds, 37 patients with sporadic SCA, and normal control subjects had alleles containing from 21 to 36 repeats; whereas 7 patients from 4 kindreds had 1 expanded allele, with size varying between 53 and 62 repeats. Expanded alleles of 53 and 56 repeats were found in 2 asymptomatic carriers from the 4 kindreds with SCA1 (at the ages of 8 and 21 years, respectively). Thus, there were 17 repeats between the size ranges of normal and SCA1 alleles. No alleles intermediate between 36 and 53 repeats was observed in this study.

Frequency of the SCA2 Expanded Allele

One hundred fifty-five patients from 80 kindreds, 37 patients with sporadic SCA, and normal control subjects had alleles containing from 12 to 31 repeats, whereas 12 patients from 5 kindreds had 1 expanded allele, with sizes varying between 42 and 47 repeats. Expanded allele of 40 repeats was found in 1 asymptomatic carrier from the 5 kindreds with SCA2 (at the age of 13 years). Thus, there were 9 repeats between the size ranges of normal and SCA2 alleles. No alleles intermediate between 31 and 40 repeats was observed in this study.

Frequency of the SCA3/MJD Expanded Allele

Eighty-four patients from 44 kindreds, 37 patients with sporadic SCA, and normal control subjects had alleles containing from 13 to 38 repeats, whereas 83 patients from 41 kindreds had 1 expanded allele, with sizes varying between 68 and 83 repeats. Expanded alleles ranged from 66 to 81 repeats were found in 23 asymptomatic carrier from the 41 kindreds with SCA3/MJD (at the ages of 7-31 years; mean age, 19.09 ± 13.07 years). Thus, there were 30 repeats between the size ranges of normal and SCA3/MJD alleles. No alleles intermediate between 38 and 63 repeats was observed in this study.

Frequency of the SCA6, SCA7, and DRPLA Expanded Allele

No expanded CAG repeat at the SCA6, SCA7, or DRPLA locus was found in all of the patients with SCA and the control subjects. The number of CAG repeats ranged from 11 to 17 at the SCA6 locus, from 8 to 19 at the SCA7 locus, from 9 to 21 at the DRPLA locus.

GENETIC ANALYSIS

Genetic DNA was isolated from peripheral leukocytes as described elsewhere.12 Polymerase chain reaction (PCR) amplification of the CAG repeat was performed using primers Rep1 and Rep2, for SCA1, SCA2, and SCA2B for SCA2, MJD25 and MJD32, for SCA3/MJD, S-5-F1 and S-5-R1 for SCA6,4 4U1024 and 4U1716 for SCA7, and CTG-B37-F and CTG-B37-R for DRPLA1 genes. All 6 alleles were amplified and analyzed on polyacrylamide gels using standard electrophoresis methods. To determine the exact CAG repeat length, we used highly denaturing electrophoresis conditions. Each polymerase chain reaction product (4 µL) was mixed with 4 µL of formamide loading buffer (98% formamide, 10 mmol/L EDTA; pH, 8.0; 0.025% xylene cyanol FF, and 0.025% bromphenol blue), denatured at 99°C for 9 minutes, then placed on ice. Gel electrophoresis was performed through an 8% polyacrylamide gel with 7-mol/L urea and 42% formamide. Electrophoresis was carried out at 1000 V, at 45°C for 5 hours. pBR322/MspI was used as a DNA size marker. The gels were silver stained.

STATISTICAL ANALYSIS

Comparisons of means were performed using the 2-tailed t test and analysis of variance test. Comparisons of frequencies were performed using the χ2 test or Yates corrected χ2 test. Pearson product moment correlation and regression coefficients were calculated to evaluate the correlation between the age at onset of SCA and the length of CAG repeat on the affected chromosomes of these patients.

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We have studied 85 Chinese kindreds with SCA and identified the SCA1 mutation in 4.70%, the SCA2 mutation in 5.88%, and the SCA3/MJD mutation in 48.23% of the overall kindreds. There are still 41.19% of our kindreds and the patients with sporadic SCA who did not test positive for any of the described SCAs. Similar researches in non-Chinese populations indicate that the frequency of SCA1, SCA2, SCA3/MJD, SCA6, and DRPLA is about 3% to 27%, 5% to 13%, 23% to 55%, 6% to 27%, and 1% to 20%, respectively. The diversity of these results is correlated with racial differentiation and dissimilar genetic backgrounds. But more accordant viewpoints of SCA are as follows: (1) SCA3/MJD is the most common genotype; (2) DRPLA is predominantly existent in the Japanese; and (3) SCA6 is more prevalent in the groups from Germany, the United States and Japan, while SCA7 is more prevalent in the United States and France. Our research suggests that the SCA3/MJD mutation seems to be the most common cause of dominantly inherited SCA in China, but SCA1 and SCA2 mutations are rare. The patients with SCA3/MJD do not have any Portuguese origin; it also indicates that Chinese patients with SCA3/MJD are non-Portuguese types; and SCA3/MJD is really a worldwide disease.

The correlation between clinical features and expanded CAG repeats or duration of diseases in patients with SCA3/MJD are listed in Table 1. The correlation between the severity of illness and the expanded CAG repeats or duration of diseases in patients with SCA3/MJD are listed in Table 2. The major clinical features of patients with SCA in different subtypes are listed in Table 3.

Table 1. Correlation Between the Clinical Features and the Expanded CAG Repeats or Duration of Disease in Patients With SCA3/MJD*

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Mean Expanded CAG Repeats</th>
<th>Mean Duration of Diseases, y</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Present (No. of Patients)</td>
<td>Absent (No. of Patients)</td>
</tr>
<tr>
<td>Swallowing difficulties</td>
<td>77.84 ± 2.79 (36)</td>
<td>75.43 ± 3.65 (47)</td>
</tr>
<tr>
<td>Nystagmus</td>
<td>76.94 ± 3.49 (51)</td>
<td>75.43 ± 3.30 (32)</td>
</tr>
<tr>
<td>Slow saccades</td>
<td>77.68 ± 3.48 (22)</td>
<td>76.00 ± 3.32 (61)</td>
</tr>
<tr>
<td>Ophthalmoplegia</td>
<td>78.53 ± 2.84 (19)</td>
<td>75.76 ± 3.41 (64)</td>
</tr>
<tr>
<td>Facial and lingual fasciculation</td>
<td>77.05 ± 3.25 (21)</td>
<td>76.18 ± 3.54 (62)</td>
</tr>
<tr>
<td>Bulging eyes</td>
<td>77.10 ± 3.11 (10)</td>
<td>76.30 ± 3.53 (73)</td>
</tr>
<tr>
<td>Spasticity</td>
<td>76.51 ± 3.58 (61)</td>
<td>76.09 ± 3.19 (22)</td>
</tr>
<tr>
<td>Hyperreflexia</td>
<td>76.48 ± 3.56 (65)</td>
<td>76.11 ± 3.22 (18)</td>
</tr>
<tr>
<td>Hyporeflexia</td>
<td>76.60 ± 4.84 (5)</td>
<td>76.29 ± 3.43 (80)</td>
</tr>
<tr>
<td>Babinski signs</td>
<td>76.55 ± 3.24 (37)</td>
<td>76.20 ± 3.67 (46)</td>
</tr>
<tr>
<td>Amyotrophy</td>
<td>78.37 ± 2.45 (19)</td>
<td>75.81 ± 3.53 (64)</td>
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* Values expressed as mean ± SD. Comparisons of means were performed with 2-tailed t test and analysis of variance test. SCA3/MJD indicates spinocerebellar ataxia type 3/Machado-Joseph disease.

Table 2. Correlation Between the Severity of Illness and the Expanded CAG Repeats or Duration of Disease in Patients With SCA3/MJD*

<table>
<thead>
<tr>
<th>Severity of Illness†</th>
<th>Mean Expanded CAG Repeats (No. of Patients)</th>
<th>Mean Duration of Disease, y (No. of Patients)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>73.58 ± 2.02 (12)</td>
<td>2.67 ± 1.87 (12)</td>
<td>.001</td>
</tr>
<tr>
<td>Grade 2</td>
<td>75.66 ± 2.65 (44)</td>
<td>7.09 ± 4.65 (44)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Grade 3</td>
<td>78.86 ± 2.39 (5)</td>
<td>12.89 ± 5.51 (27)</td>
<td>.35</td>
</tr>
</tbody>
</table>

* Values expressed as mean ± SD. Comparisons of means were performed with 2-tailed t test and analysis of variance test. †Grade 1 indicates the patient can walk independently or occasionally needs help; grade 2, patient completely needs support; and grade 3, patient is only wheelchair bound or bedridden.17

We have studied 85 Chinese kindreds with SCA and identified the SCA1 mutation in 4.70%, the SCA2 mutation in 5.88%, and the SCA3/MJD mutation in 48.23% of the overall kindreds. There are still 41.19% of our kindreds and the patients with sporadic SCA who did not test positive for any of the described SCAs. Similar researches in non-Chinese populations indicate that the frequency of SCA1, SCA2, SCA3/MJD, SCA6, and DRPLA is about 3% to 27%, 5% to 13%, 23% to 55%, 6% to 27%, and 1% to 20%, respectively. The diversity of these results is correlated with racial differentiation and dissimilar genetic backgrounds. But more accordant viewpoints of SCA are as follows: (1) SCA3/MJD is the most common genotype; (2) DRPLA is predominantly existent in the Japanese; and (3) SCA6 is more prevalent in the groups from Germany, the United States and Japan, while SCA7 is more prevalent in the United States and France. Our research suggests that the SCA3/MJD mutation seems to be the most common cause of dominantly inherited SCA in China, but SCA1 and SCA2 mutations are rare. The patients with SCA3/MJD do not have any Portuguese origin; it also indicates that Chinese patients with SCA3/MJD are non-Portuguese types; and SCA3/MJD is really a worldwide disease.

On the study of CAG repeat length in the expanded allele of patients with SCA3/MJD, we find there is a significant negative correlation between the size of the CAG repeat and the age at onset (r = −0.70, P < .001); the larger CAG repeats is, the earlier onset age concomitants (Figure 1, left). From Table 1 and Table 2, we could discover that there is a positive correlation between expanded CAG repeats of patients with SCA3/MJD and severity of illness. In addition, the expanded CAG repeats of patients with SCA3/MJD also correlate with some symptoms, such as swallowing difficulties, ophthalmoplegia, and amyotrophy. But the severity of illness and the appearance of clinical signs are affected by the duration of disease; larger CAG repeats and longer disease duration usually accompany a more severe illness. In our research, the expanded CAG repeats of patients with SCA3/MJD have no significant variation between paternal and maternal transmission; this disagrees with other reports.17,18 However, some younger members of the kindreds are seen with a predominant maternal transmission.

We could not determine the correlation between the expanded CAG repeats and clinical symptoms, severity
of illness, or sex of the parent transmitting the mutation in patients with SCA2 because of the few patients studied. But there is also a significant negative correlation between expanded CAG repeats and age at onset of patients with SCA2 ($r = -0.83, P < .01)$ \(^{19}\) (Figure 1, right).

Owing to the limited quantity of SCA1, we do not make any further analysis. Further research including more kindreds might help us find out the correlation between expanded CAG repeats and clinical features of patients with SCA1. In a word, our results are similar to other reports. \(^{1,3,4,17,20,22}\) We can confirm that there exists a negative correlation between the expanded CAG repeats and the age at onset, and a positive correlation between the expanded CAG repeats and the severity of illness. But the relativity of clinical symptoms and CAG repeats in different research studies is conflicting, which indicates that duration of disease is also a main influencing factor on clinical features. \(^{22}\)

From Table 3, the common manifestations of patients with SCA in this group include progressive cerebellar ataxia and dysarthria. Besides this, patients with SCA1 also frequently have swallowing difficulties and hyporeflexia, but no extrapyramidal symptoms or dementia. Most patients with SCA2 have hyporeflexia, swallowing difficulties, dementia, and amyotrophy, while few have slow saccades, ophthalmoplegia, pyramidal signs,
and chorea. Furthermore, hyporeflexia and dementia might be helpful in clinical work to distinguish patients with SCA2 from others with different types of SCA. In patients with SCA3/MJD, spasticity, hyperreflexia, Babinski signs, nystagmus, and swallowing difficulties are frequent; slow saccades, ophthalmoplegia, facial and lingual fasciculation, and amyotrophy are next; but chorea, dementia, and hyporeflexia are rare. Furthermore, spasticity, hyperreflexia, and Babinski signs might be helpful in clinical work to distinguish patients with SCA3/MJD from others with different types of SCA. In patients with untyped SCA, nystagmus and hyporeflexia are more frequent, while slow saccades, facial and lingual fasciculation, hyporeflexia, amyotrophy, chorea, and dementia are less frequent. In patients with sporadic SCA, hyperreflexia and spasticity are more frequent, while slow saccades, facial and lingual fasciculation, amyotrophy, and chorea are less frequent. In addition, we also discovered that nystagmus (P<.01), slow saccades (P<.05), and facial and lingual fasciculation (P<.05) are more frequent in patients with autosomal dominant compared with patients with sporadic SCA. In comparison with other studies, 1,2,5,14,16 these differences reflect racial differentiation and dissimilar genetic backgrounds, and probably may also be associated with fewer cases (SCA1 and SCA2) and different disease duration in our study.

Nevertheless, the hereditary SCAs are phenotypically and genotypically heterogeneous. More refined clinical classification of the SCAs has been hampered by the marked variation in phenotypes that is observed even within families. Only genetic classification can determine the final diagnosis in patients with SCA. Therefore, gene diagnosis and genetic classification are important for the clinical study of SCA. We are carrying out experiments on gene location and genetic classification in our patients with untyped hereditary SCA.

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