Molecular and Clinical Study of Spinocerebellar Ataxia Type 7 in Chinese Kindreds

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Objective: To investigate the clinical and molecular characteristics of spinocerebellar ataxia type 7 (SCA7) in Chinese kindreds.

Background: Spinocerebellar ataxia type 7 is caused by the expansion of an unstable CAG repeat in the first exon of the SCA7 gene.

Methods: Clinical and related examinations were performed in all affected or at-risk individuals from 4 Chinese families presenting with autosomal dominant cerebellar ataxia and decreased visual acuity. The size of the (CAG)n array of the SCA7 gene was detected by polymerase chain reaction, polyacrylamide gel electrophoresis, and related techniques in the 4 families and 67 healthy controls. The relationship between expanded repeat number and age of onset was statistically analyzed.

Results: The SCA7 mutation was identified in 2 families. Clinical study revealed that great variation occurred in the age of onset, initial symptoms, and associated signs. Meanwhile, the analysis of 11 parent-child couples demonstrated the existence of marked anticipation. Some distinct retinal changes were noted in 2 affected patients. All SCA7 patients in our series exhibited expanded CAG repeats, ranging from 44 to 85 repeats, with a strong negative correlation between repeat size and age of onset. Repeat lengths of expanded alleles showed somatic mosaicism in leukocyte DNA. There were some subtle clinical differences between the SCA7-positive and -negative cases.

Conclusions: Clinical variation occurred not only among the SCA7 families but also within the same kindred. Meiotic and mitotic instability of the CAG repeat in the SCA7 gene were demonstrated, and intergenerational instability of the array was associated with the clinical phenomenon of anticipation.

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SUBJECTS AND METHODS

SUBJECTS

A total of 25 affected or at-risk individuals from 4 kindreds presenting with autosomal dominant SCA and decreased visual acuity and 67 healthy Chinese volunteers were selected for this study. Clinical examinations were performed by a neurologist (G.W.) and an ophthalmologist (Y.W.). All members of the families underwent a complete general neurologic examination, particularly of the extraocular muscle and of cerebellar function, and an ophthalmologic examination, including visual acuity, color discrimination, and direct ophthalmoscopy. Macular and retinal photographs were taken and electroretinography was performed in some patients. A detailed family history was obtained through personal interviews with available family members.

ANALYSIS OF NUMBERS OF CAG REPEAT UNITS

Genomic DNA was extracted from peripheral leukocytes. The SCA7 gene repeat expansion assay was done with SCA7-F1 (5’-TTTTTTTTATTTTAGGACG-3’) and SCA7-R1 (5’-CCTTCAG-GACTGGGCAAG-3’), as outlined by Koob et al.8 Polymerase chain reactions (PCRs) were performed in a total volume of 25 µL containing 200 ng of genomic DNA; 7.5 pmol of each primer; dideoxyadenosine triphosphate, dideoxyguanosine triphosphate, and dideoxythymosine triphosphate, each at 200 µmol/L; 30-µmol/L dideoxycytosine triphosphate; 370 kBq of phosphate 32 dideoxycytosine triphosphate; 10-mmol/L Tris hydrochloride (pH 9.0); 50-mmol/L potassium chloride; 0.1% Triton X-100; 1.5-mmol/L magnesium chloride; 8% dimethyl sulfoxide; and 3 U of Taq polymerase (Sino-American Biotechnology Co, Luoyang, China). Genomic DNA was first denatured at 95°C for 2 minutes, and then 35 cycles at 94°C for 50 seconds, 55°C for 75 seconds, and 72°C for 1 minute were performed on a PCR system (Gene Amp; Perkin-Elmer Analytical Instruments, Norwalk, Conn), followed by a final extension step of 2 minutes. The PCR products were denatured and electrophoresed on a 6% denaturing polyacrylamide gel along with PCR products from cloned DNAs of normal and expanded alleles as molecular size standards and then subjected to autoradiography.

STATISTICAL ANALYSIS

Correlation of the age of onset with the expanded (CAG)n array size was determined using the Pearson correlation coefficient. Other statistical analyses were performed using the Student t test.

failure preceded ataxia in all patients of family A and 2 patients of family B, whereas in the remainder, it was noted that the 2 symptoms appeared together. Besides ataxia and visual loss, external ophthalmoplegia of various degrees, marked slowing of saccades, variable retinal impairment, and pyramidal sign were observed. During the examination of color discrimination, the D15 colorchip (Chang Feng Medical Instruments, Beijing, China) was more sensitive than pseudoseichromatic plates in detecting an abnormality, especially of the tritan axis, at the early stage of the disease. We describe 2 patients with different manifestations illustrating the range and clinical variation.

Patient B:IV-10

The proband of family B first noted unsteadiness during running at age 5 years, and within 2 years, this had progressed to affect walking. Around this time, she developed blurring of vision and speech difficulty. Physical examination at age 7 showed visual acuity without correction to be limited to counting fingers at 30 cm so that color discrimination and visual field defects could not be detected. Pupils were 8 mm in darkness and 7 mm in light bilaterally. Extraocular movements were impaired, especially up gaze and convergence. Saccades were very slow in all directions while pursuit was fair. Nystagmus was absent. Slitlamp examination disclosed a normal anterior segment. The fundus showed scattered dust-like pigmented degeneration in the peripheral retina and comparatively normal appearance at the macula, with disc pallor and arterial attenuation (Figure 2, A). Speech was severely dysarthric. There was mild weakness with increased muscle tone. Plantar responses were equivocal bilaterally. Responses to finger-nose and heel-to-shin tests were slow and ataxic, and rapid alternating movements...
were markedly impaired. Periodic slight head tremor was noted. Sensation remained normal.

**Patient B:IV-4**

The female cousin of patient B:IV-10 had a relatively benign course. Visual impairment was noted at age 10 years and gradually progressed. At age 11, she developed dizziness. Examination at age 13 demonstrated visual acuity of 20/200 OU that could be corrected to 20/160 OU bilaterally. Color plate testing revealed that she was unable to see any color. Ophthalmoscopy showed mottling of pigment at the macula and mildly scattered dust-like pigmentary degeneration in the peripheral retina with disc pallor (Figure 2, B). Visual fields revealed concentric narrowing (Figure 2, C). Scotopic electroretinography showed prolonged latency and decreased amplitude of b-waves in the right eye and absent a- and b-waves in the left eye (Figure 3). Extraocular motility was approximately fair except for impaired convergence. Saccades were slow but pursuit movements were normal. Power and tone were normal while reflexes were brisk bilaterally. Plantar reflexes were normal. Response to the finger-nose test was fair, but the response to the heel-to-shin test was not smooth, and rapid alternating movements were slow.

Meanwhile, the patients of families C and D without the SCA7 mutation also manifested with cerebellar ataxia and visual loss, but there were some fine differences between the SCA7-positive and -negative cases, as follows: SCA7 patients had more typical anticipation and retinal pigmentary degeneration, whereas nystagmus was more common in the other patients. However, definite clinical discrimination requires further pedigree collection. Clinical features of the 12 fully examined patients are summarized in the Table.

**ANALYSIS OF THE NUMBER OF CAG REPEATS AND CORRELATION BETWEEN NUMBER AND CLINICAL FEATURES**

Eight patients with the SCA7 phenotype and a 3-year-old girl with normal presentation (patient A:IV-3) were heterozygous for alleles with the (CAG)_n array within a range of 44 to 85 repeats (Table). The sizes of normal alleles of the Chinese control subjects fell within the range of 9 to 18 repeat units, with a 10-repeat allele accounting for 78% of the normal chromosomes. There was no intermediate allele observed in our series. The...
electrophoretic pattern of the expanded alleles (Figure 4) suggested that significant somatic mosaicism was present in the SCA7 patients, which was confirmed by sequence analyses. This phenomenon was also observed in other SCAs.9,10 There was a strong negative correlation \( r = -0.883 \) between the age at onset and the size of the CAG repeat number (Figure 5). A mean increase of 13 CAG repeats per transmission was observed in 6 parent-child pairs, including 1 paternal (increase of 37 CAG repeats) and 5 maternal (mean increase of 8 CAG repeats) transmissions. Patient B:IV-10, with an age at onset of 5 years, had the largest expansion of 85 repeat units inherited from her father and presented with periodic slight head tremor and the most severe clinical course in our series.

Normal SCA7 alleles contained 9 to 18 repeats with low polymorphism, whereas the pathological alleles carried 44 to 85 repeats with great instability. There was a strong negative correlation \( r = -0.883 \) between the age at onset and the size of the CAG repeat expansion in Chinese SCA7 patients, and a longer (CAG)\(_n\) array seemed to be associated with a more severe clinical course. These observations are consistent with those in previous studies of other populations.13-15 All transmissions of the pathological alleles showed an increase in the CAG repeat length. The degree of intergenerational instability of the CAG repeat is greater in SCA7 than in any of the other 7 known neurodegenerative diseases caused by a translated CAG repeat expansion.6 Somatic mosaicism revealed by electrophoresis and sequence analyses suggests mitotic instability of the trinucleotide repeats located on the expanded alleles. Mosaicism in the brain remains to be investigated. The mechanism of instability of the CAG repeats is unknown. In SCA3/Machado-Joseph disease, polymorphisms located close to the repeats seem to influence the degree of the instability.16,17 but none of these polymorphisms was found in the PCR products of our study from the 2 pathogenic alleles and 3 normal alleles that were sequenced. Two juvenile patients (B:IV-10 and B:IV-4) inherited the disease alleles from the affected father and mother, respectively, and the paternal transmission resulted in a more severe clinical course. Although the dynamic repeat expansion from the paternal transmission was greater than those from the maternal transmissions, maternal transmission of the disease was more common. Gouw et al16 also reported this observation, suggesting germ-line or embryonic effects of the repeat expansion. It is

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**Clinical Features and Numbers of CAG Repeats of SCA7-Positive and -Negative Patients**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>A:III-4</th>
<th>A:III-5</th>
<th>B:II-5</th>
<th>B:III-1</th>
<th>B:III-4</th>
<th>B:III-10</th>
<th>B:IV-4</th>
<th>B:IV-10</th>
<th>C:II-3</th>
<th>C:III-3</th>
<th>D:II-2</th>
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<td>Peripheral pigmentary degeneration</td>
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<tr>
<td>Dystonia</td>
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<td>Decreased vibration sense</td>
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<tr>
<td>Slight tremor</td>
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<td>–</td>
</tr>
<tr>
<td>No. of CAG repeats†</td>
<td>52/12</td>
<td>47/11</td>
<td>44/12</td>
<td>50/10</td>
<td>52/12</td>
<td>48/10</td>
<td>65/9</td>
<td>85/12</td>
<td>12/10</td>
<td>10/10</td>
<td>15/11</td>
<td>16/10</td>
</tr>
</tbody>
</table>

*SCA7 indicates spinocerebellar ataxia type 7; plus sign, positive; minus sign, negative; +/-, dubious; and /, color discrimination could not be examined because of poor visual acuity.

†Polymerase chain reaction products. Sequence analysis revealed somatic mosaicism of CAG repeats carried by the following pathogenic alleles: B:II-5, 45/44; and B:IV-10, 85/84.

The results of our molecular analyses confirmed the clinical diagnoses of SCA7 for 2 families, and the other 2 kindreds with the SCA7 phenotype did not have the CAG expansion that indicated locus heterogeneity. The analyses of the clinical features showed marked variability in age at onset, initial symptoms, and disease duration. Patient B:IV-10, who had the earliest age of onset and most severe clinical course, presented with pigmentary degeneration in the peripheral retina and a relatively normal macula. Patient B:IV-4, with pigmentary degeneration both at the macula and at the peripheral retina, presented with concentric narrowing of the field of vision. These features are different from those of most previously reported SCA7 patients with predominant maculopathies and central scotoma.3,11,12

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worth mentioning that at the time of this study, patient A:IV-3, another juvenile with normal clinical presentation, had an expanded (CAG)n array of 57 repeats—perhaps she had not yet come to the age at onset of the disease. In conclusion, our research demonstrates that the SCA7 gene in the Chinese population is genetically homogeneous with other populations. Molecular testing of the SCA7 gene might enable us to delineate the clinical spectrum of SCA7, and it might be helpful for differential and presymptomatic diagnosis.

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