Frequency Analysis of Autosomal Dominant Cerebellar Ataxias in Taiwanese Patients and Clinical and Molecular Characterization of Spinocerebellar Ataxia Type 6

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Background: Spinocerebellar ataxia (SCA) is a heterogeneous group of neurodegenerative disorders. The mutational basis for most of these disorders is an expanded CAG repeat sequence within the coding regions of the genes involved. The prevalence of SCA in the ethnic Chinese on Taiwan remains unclear. Moreover, there has been no report of SCA type 6 (SCA6) among Chinese people.

Objectives: To characterize the prevalence of SCA in the ethnic Chinese on Taiwan, and to specifically characterize Chinese patients with SCA6 in terms of clinical and molecular features.

Patients and Methods: Using a molecular approach, we investigated SCA in 74 Taiwanese families with dominantly inherited ataxias and in 49 Taiwanese patients with sporadic ataxias. Clinical and molecular features of SCA6 were further characterized in 12 patients from 8 families and in 2 sporadic cases. Furthermore, the intragenic polymorphic marker D19S1150 was amplified by polymerase chain reaction to analyze for linkage disequilibrium.

Results: Machado-Joseph disease–SCA3 was the most common type of autosomal dominant SCA in the Taiwanese cohort, accounting for 35 cases (47.3%), followed by SCA6 (8 [10.8%]), SCA2 (8 [10.8%]), SCA1 (4 [5.4%]), SCA7 (2 [2.7%]), dentatorubropallidoluysian atrophy (1 [1.4%]), and SCA8 (0%). The genes responsible for 16 (21.6%) of Taiwanese dominantly inherited SCA cases remain to be determined. Among the 49 patients with sporadic ataxias in the present series, 2 (4.1%) were found to harbor SCA6 mutations. In the families with SCA6, we found significant anticipation in the absence of genetic instability on transmission, indicating that some other mechanism might account for the anticipation. The same frequent allele of the intragenic DNA marker (D19S1150) was shared by 7 of 10 Taiwanese families with SCA6.

Conclusions: Although SCA6 has, so far, not been reported in mainland Chinese, we found a geographic cluster of families with SCA6 on Taiwan. Genotyping studies suggest a founder effect in the Taiwanese patients with SCA6.

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SUBJECTS AND METHODS

SUBJECTS

Blood samples were obtained from 200 unrelated healthy Taiwanese volunteers, 49 Taiwanese patients with idiopathic sporadic ataxias, and 140 Taiwanese individuals from 74 unrelated families with dominantly inherited ataxias. All of them were Han Chinese, whose families had emigrated from mainland China at different times during the past 400 years. Patients with ataxia caused by abuse of alcohol or other substances, paraneoplasia, malformation, vascular defects, inflammation, or autoimmune diseases were excluded. Twelve affected individuals from 8 families and 2 patients with sporadic ataxia who had SCA6 CAG expansions underwent further clinical evaluations by a board-certified neurologist (B.S.). Age at onset was provided by the patient or close relatives. Informed consent was obtained from all subjects before participation in the study.

MOLECULAR STUDIES

Genomic DNA was isolated from peripheral leukocytes as previously described.\textsuperscript{17,18} Polymerase chain reaction (PCR) was performed with the primers Rep1 and Rep2 for SCA1,\textsuperscript{7} F-1 and R-1 for SCA2,\textsuperscript{7} MJD2\textsuperscript{5} and MJD3\textsuperscript{2} for MJD-SCA3,\textsuperscript{9,19} S-5-F1 and S-5-R1 for SCA6,\textsuperscript{10} 4U1024 and 4U716 for SCA7,\textsuperscript{11} SCA8-F4 and SCA8-R4 for SCA8,\textsuperscript{12} and CTG-B37 primer sets for DRPLA.\textsuperscript{13} The PCR conditions were as described in each original report. Alleles were separated by means of electrophoresis on 6% polyacrylamide gels in parallel with an M13 sequencing ladder and were analyzed as previously described.\textsuperscript{17,19} Reaction mixtures from related family members were run in adjacent lanes.

To accurately assess the size of the alleles, we sequenced at least 2 independent clones for each allele in the patients with SCA. The genomic DNA was amplified with each of the primer sets, and then subcloned and sequenced. Sequencing reactions were performed with a DNA sequencing kit (Sequenase version 2.0; United States Biochemical, Cleveland, Ohio).

The (CA)\textsubscript{n} microsatellite marker D19S1150 (Genome Database; available at: http://www.gdb.org) was amplified by means of PCR to analyze for possible linkage disequilibrium.\textsuperscript{20-22} The allele lengths of the marker D19S1150 were defined by alignment with a sequencing ladder (allele 5/8/9: 156/162/164 base pairs).

STATISTICAL ANALYSIS

Statistical analyses were performed with SAS software (SAS Institute Inc, Cary, NC). The null hypothesis was rejected for $P<.05$. Group data were compared with the Wilcoxon rank sum test or the $\chi^2$ test. Data are given as mean ± SD.

RESULTS

FREQUENCY ANALYSIS OF SCA

The numbers of unrelated kindreds with positive test results were 4 for SCA1, 8 for SCA2, 35 for SCA3, 8 for SCA6, 2 for SCA7, and 1 for DRPLA. The prevalence of MJD-SCA3 in the 74 Taiwanese families with autosomal dominant SCA was 47.3% (n = 35), followed by SCA6 (10.8% [n = 8]), SCA2 (10.8% [n = 8]), SCA1 (5.4% [n = 4]), SCA7 (2.7% [n = 2]), and DRPLA (1.4% [n = 1]) (Table 1). Of the families with dominantly inherited SCA, 16 (21.6%) did not harbor any of the above 7 mutations. Among the 49 patients with sporadic ataxias who underwent gene testing, 2 (4.1%) were found to harbor SCA6 mutations.

CLINICAL FEATURES OF SCA6

The main clinical features of the 14 individuals affected with SCA6 in this study are summarized in Table 2. There were 8 men and 6 women, with an age range of 44 to 79 years (59.6±10.8 years) and duration of symptoms ranging from 4 to 29 years (11.6±7.7 years). No significant differences were found in the age at onset between the men (49.1±8.3 years) and women (46.5±6.7 years) ($P = .79$). In pedigree 5, subjects 5, 6, 8, and 9 were siblings (Table 2). Subject 7 was the eldest daughter of subject 6. Despite an identical number of repeats (ie, 23), there was a mild to moderate degree of variability in age at onset (42-52 years) and duration of illness (5-26 years) among the members of the same generation in this family.

In the 9 parent-child pairs for whom the ages at onset for both were known, the mean anticipation was 6.8±9.8 years (range, -7 to 25 years; $P = .04$). Anticipation was not statistically comparable between paternal (25 years; n = 1) and maternal (4.5±7.4 years; n = 8) transmission in this series because of the availability of only 1 case of paternal transmission.

GENETIC STUDIES OF SCA6

Normal alleles (n=400) ranged from 5 to 18 repeat units (12.4±1.9 repeat units), with the most frequent alleles being 13 (42.9%), 14 (17.2%), and 11 (17.2%) repeat units. No significant differences were found in the age at onset between normal and pathologic alleles. Review of the family history in the 2 patients with sporadic ataxias showed that the mother of subject 10 died at the age of 35 years of “depression” and the mother of subject 13 died at the age of 38 years during childbirth. None of the parents of these 2 patients presented with movement abnormalities, nor were cerebellar symptoms detected in any of the relatives of these patients.

In contrast to the instability of SCA1, SCA2, and MJD-SCA3 expanded alleles, the SCA6 expanded allele appeared quite stable. There were 17 individuals (1 affected and 16 asymptomatic) who had expanded alleles and for whom data on the expanded alleles in the af-
The same allele, 8, of the intragenic marker D19S1150 was present on 7 (70%) of the disease-bearing chromosomes in the 10 Taiwanese families with SCA6 with different numbers of CAG repeats (n=21, 22, 23, and 25). The frequency of the same allele in the control population was 26.7% (P < .01). Two other families shared another common allele, 5, on their disease-bearing chromosomes.

Statistical analysis of the correlation between CAG trinucleotide repeat length and age at onset was not possible because of the small sample size, narrow range of expanded allele size (21-25 repeat units), low variability of the expanded alleles (78.6% of expanded alleles consisted of 23 CAG repeats), scattering of ages at onset defined by a single repeat number, and lack of transmission instability in the limited number of meioses studied.

This study found an important difference in the prevalence of SCA between Taiwanese and mainland Chinese populations. In the Chinese population on both sides of the Taiwan Strait, as in white and Japanese populations, MJD-SCA3 mutation was the most common cause of SCA.23 Second to MJD-SCA3, SCA6 was the most common dominantly inherited cerebellar ataxia in Taiwanese families, as it was in Japanese (11%),23,25 but not in cases of inherited cerebellar ataxias in white (5%)23 or mainland Chinese (Guo-Xiang Wang, MD [wang06@public.hz.com.cn], e-mail, January 17, 2000)16 patients. The molecular basis for the differences in the prevalence of these dominant SCAs is not fully understood. It has been suggested that the relative prevalences of the dominant SCAs are determined by the balance between the continuous generation of new expanded alleles and the loss of expanded alleles that is due to the impaired reproduction fitness of severely affected patients.23 Thus, the high frequency of SCA6 mutation in Taiwanese and Japanese persons might be partly accounted for by the greater frequencies of healthy individuals with CAG repeats larger than 13 in Taiwanese (19.8%) and Japanese (20%) populations than that reported in white populations (4%).10,23,26 This may occur because some of the large normal alleles stochastically undergo expansion mutations to produce the new expanded alleles.23 However, this seems unlikely in view of the meiotic stability of SCA6. Alternatively, it could be due to a founder effect.21,23,27-29

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**Table 1. Frequency Distribution of Spinocerebellar Ataxia Subtypes in Different Ethnic Populations**

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>No. of Subjects</th>
<th>SCA1</th>
<th>SCA2</th>
<th>SCA3</th>
<th>SCA6</th>
<th>SCA7</th>
<th>SCA8</th>
<th>DRPLA</th>
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<td>NT</td>
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<td>22</td>
<td>Silveira et al24</td>
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<td>43</td>
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<td>NT</td>
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<td>NT</td>
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<td>41</td>
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</tr>
<tr>
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<td>22</td>
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*SCA indicates spinocerebellar ataxia; DRPLA, dentatorubro-pallidoluysian atrophy; and NT, not tested.

**Table 2. Clinical Features of 14 Taiwanese Patients With SCA6**

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<td>W</td>
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</table>

*SCA indicates spinocerebellar ataxia; Sp, sporadic; NA, not applicable; +, present/abnormal; ++, moderately abnormal; ++++, markedly abnormal; −, absent; OCN, optokinetic nystagmus; DTRs, deep tendon reflexes; A, still independently ambulating; S, support from another person needed when walking; and W, wheelchair bound.
Genotyping of Taiwanese patients with intragenic DNA marker D19S1150 on chromosome 19p13 demonstrated a shared allele of a marker within the CACNL1A4 gene in the majority (70%) of Taiwanese patients with SCA. In conjunction with the geographic clustering of the families with SCA6, this observation seems to support the hypothesis of a founder effect.22 Similar geographic clusters of SCA6 have also been observed in the Chugoku area of western Japan29 and in the North Rhine-Westphalia area of Germany.22

The predominant clinical feature of our patients with SCA6 (12 familial and 2 sporadic) was cerebellar ataxia (loss of balance and dexterity of handwriting) with an onset late in adult life and a very slowly progressive disease course (Table 2). Although brisk deep tendon reflexes were frequently observed, plantar response was normal in all of our patients, indicating that the upper motoneurons were only mildly affected.28 Other noncerebellar features, eg, rigidity, Gegenhalten, intellectual impairment, and sphincter disturbances, were rarely found in our patients with SCA6. One of our patients (subject 1) had a partial right abducens palsy and exhibited a horizontal diplopia on looking toward the right side. Many of our patients also had an exacerbation of the sense of imbalance in a visually “busy” environment, as has been previously reported by others (Sub H. Subramony, MD [s_h_s@hotmail.com], e-mail, May 10, 1999). Clinical features associated with other disorders caused by mutations in the CACNL1A4 gene,20 including migraines, episodes of hemiplegia, and ataxia, were checked carefully but rarely found in our cohort with SCA6, which is consistent with the findings of Matsumura et al25 and Gomez et al.30 The mean age at onset (48.0 ± 7.5 years) in our 14 patients with SCA6 was significantly greater than that of the other patients with SCA (eg, 33.6 ± 11.6 years in 25 patients with SCA3; P < .001).10 However, the differences between patients with SCA6 and SCA3 in terms of either anticipation (6.8 ± 9.8 vs 7.8 ± 7.7 years) or duration of illness (11.6 ± 7.7 vs 8.7 ± 5.7 years) were not statistically significant (P > .05). In all cases in this study, the disease had an indolent course, rarely progressing to severe disability within the first 10 years.

In this study we found significant anticipation (6.8 ± 9.8 years) in the absence of change in trinucleotide repeat number in Taiwanese families with SCA6, indicating that some other mechanisms accounted for the anticipation. One likely possibility seemed to be ascertainment bias, eg, that affected offspring recognize the manifestations of the disease at an earlier age because they have observed similar manifestations in the affected parent.31 Significant anticipation in the absence of changes in the number of repeats has also been observed in French32 and Japanese33 kindreds.

This series included 17 parental transmissions of the CAG repeats in the SCA6 gene. The mutant allele size remained unchanged in all of them. In the other SCAs, the expansions were always higher on paternal transmissions than on maternal transmissions.32 However, we did not have the opportunity to observe many paternal transmissions of SCA6. To date, more than 100 parent-child transmissions of SCA6 have been evaluated32,21,28,29,33,34 and only 2 expansion events (24 expanded to 26 and 20 expanded to 25) have been observed, one in a father-son pair32 and the other in a father-daughter pair.33 Thus, meiotic instability is not a prominent feature of SCA6, in contrast to SCA1,3 SCA2,8,33 MJD-SCA3,19,25,36 SCA7,13 and DRPLA,37 where approximately 70% of the expanded alleles are unstably transmitted.19,36,38-40

In conclusion, we used PCR to estimate the relative frequency of the various heritable dominant ataxias in Taiwanese. We confirmed previous data indicating that MJD-SCA3 was the most frequent SCA worldwide; DRPLA was very rare outside Japan. A substantial proportion of inherited ataxia cases were not explained by the currently known mutations. There was a higher prevalence of SCA6 in Asian populations, including Japanese and Taiwanese, compared with white populations. A strong linkage disequilibrium of intragenic DNA marker with SCA6 was found in Taiwanese, which was presumably due to a founder effect.

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