The SCA12 Mutation as a Rare Cause of Spinocerebellar Ataxia

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Background: Spinocerebellar ataxias are a group of phenotypically and genetically heterogeneous disorders characterized by progressive degeneration of the cerebellum. The expansion of a CAG repeat upstream of the PP2APR55β gene has been recently reported as a novel cause of a dominantly inherited ataxia (SCA12) in a kindred with limb tremor as an early feature.

Objective: To explore the relative frequency of SCA12 among familial and sporadic spinocerebellar ataxias in an ethnically diverse patient population.

Methods: We used polymerase chain reaction to analyze CAG repeat size in a series of patients presenting to an ataxia clinic in California.

Results: The SCA12 expansion was not detected in any of the cases investigated. The largest allele found had 22 repeats, a finding within the proposed nonpathogenic range. Distribution of repeat size and heterozygosity were similar to that described previously.

Conclusions: These results, coupled with findings in other populations, indicate that the SCA12 mutation is a rare cause of spinocerebellar degeneration. Diagnostic testing for SCA12 should be considered in patients with cerebellum disorders and an atypical clinical phenotype, especially when tremor is initially present.

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The spinocerebellar ataxias (SCAs) are a heterogeneous group of inherited neurodegenerative disorders that primarily affect the cerebellum, brainstem, and spinal tracts. At least 13 genetic loci and 7 different SCA genes have been identified. SCA1, SCA2, SCA3/MJD, SCA6, and SCA7 are caused by expansions of CAG nucleotide repeats in coding regions of the corresponding genes. However, the genetic defect underlying a significant subset of SCAs remains to be elucidated.

Recently, Holmes et al described a large pedigree (family R) with a novel form of autosomal dominant ataxia (SCA12) that was associated with the expansion of a CAG repeat that lies upstream of the gene encoding a brain-specific regulatory subunit of protein phosphatase 2A (PPP2R2B) on chromosome 5q31-33. The number of CAG repeats was 7 to 28 in healthy controls and 66 to 78 in affected family members. Although patients with the SCA12 mutation displayed clinical features similar to those found in many SCAs, the syndrome was somewhat unusual in that it presented initially with upper extremity tremor and led to dementia in some cases. No instance of expansion was detected in 394 neurologically normal subjects and 1099 individuals with neurologic disease, including 748 subjects with ataxia, suggesting that SCA12 is uncommon among spinocerebellar degenerations. Most subjects studied were of European ancestry. It is, therefore, important to screen additional series of patients for expansions to determine the relative prevalence of SCA12 among ataxias. Herein, we investigated the SCA12 mutation in an ethnically heterogeneous group of patients with ataxia.

RESULTS

One hundred eighty kindreds were screened for the SCA12 mutation in the present study, including families of Chinese, Japanese, Southeast Asian, East Indian, Middle Eastern, Hispanic, African American, and European ethnic origin. There were 96 patients (45%) with a domi-
METHODS

We examined 211 patients from 180 families of diverse ethnic background with inherited or sporadic ataxia. All subjects were ascertained for spinocerebellar degeneration in the University of California, Los Angeles, Neurological Services ataxia clinic. Cases were classified by mode of inheritance and subdivided according to clinical features; those with an autosomal dominant pattern of inheritance were categorized using Harding’s classification. After obtaining informed consent, genomic DNA was purified from peripheral blood leukocytes using the Puregene kit (Gentra Systems, Plymouth, Minn). The SCA12 repeat was amplified by polymerase chain reaction (PCR) in 20 µL of total volume reactions containing 40 ng of DNA; 1pM of IRD-700–labeled primer (5’TGCCTGGGAAAGATCGTG-3’); 10pM of unlabeled primer (5’GCCAGGCACCTCACCCTC-3’); 250µM of deoxynucleosine triphosphate, deoxyctydine triphosphate, deoxymyidine triphosphate, and deoxadenosine triphosphate; 1× PCR buffer containing 1.5mM magnesium chloride; 1× Q-Solution (Qiagen). After an initial denaturation step of 5 minutes at 95°C, 33 cycles of PCR were performed at 95°C for 5 seconds, 60°C for 30 seconds, and 72°C for 45 seconds, followed by a final extension at 72°C for 10 minutes. The PCR products were loaded on 6% acrylamide gels and electrophoresed in an automated sequencer (LI-COR, Inc, Lincoln, Neb) at 1500 V for 4 hours at 50°C. Fragment sizes were determined with GenetmagLR computer software version 3.0 (LI-COR, Inc). The number of CAG repeats was calculated using the following formula:

$$\text{Number of Repeats} = \frac{(\text{Base Pairs} - 122)}{3}$$

nant family history, 97 patients (46%) with a recessive or sporadic ataxia, and 18 patients (9%) in whom the mode of inheritance could not be determined. The Table shows the patient phenotypic distribution.

The PCR amplification of the SCA12 locus gave a pattern of 2 distinct alleles in most cases analyzed. Under the conditions used, the presence of spurious stutter bands was minimized and did not interfere with geno-
type interpretation. Allele sizes ranged from 9 to 22 CAG repeats, with (CAG)10 being the most frequently encountered (Figure). Heterozygosity was 61.6%.

The SCA12 expansion was not detected in any of the patients studied. A control sample with known allele sizes of 10 and 78 CAG repeats was included in all experiments. The genotype of this sample showed clearly visible alleles of the expected size, therefore minimizing the possibility that expanded alleles in this size range were missed because of inefficient amplification.

**Classification of Ataxia Patients**

<table>
<thead>
<tr>
<th>Type of Ataxia</th>
<th>No. (%) of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant</td>
<td></td>
</tr>
<tr>
<td>ADCA I</td>
<td>69 (33)</td>
</tr>
<tr>
<td>ADCA II</td>
<td>4 (2)</td>
</tr>
<tr>
<td>ADCA III</td>
<td>23 (11)</td>
</tr>
<tr>
<td>Recessive or sporadic</td>
<td></td>
</tr>
<tr>
<td>Cerebellar, brainstem</td>
<td>32 (15)</td>
</tr>
<tr>
<td>Predominantly cerebellar</td>
<td>34 (16)</td>
</tr>
<tr>
<td>Friedreich phenotype</td>
<td>9 (4)</td>
</tr>
<tr>
<td>Multisystem atrophy</td>
<td>13 (6)</td>
</tr>
<tr>
<td>Spastic paraparesis</td>
<td>9 (4)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>18 (9)</td>
</tr>
<tr>
<td>Total</td>
<td>211 (100)</td>
</tr>
</tbody>
</table>

*ADCA indicates autosomal dominant cerebellar ataxia.

**COMMENT**

An expansion of a novel CAG repeat on chromosome 5 has been associated with spinocerebellar ataxia in a kindred of German descent. Recently, a second family of different ethnic origin with a similar phenotype was identified as having SCA12 expansions between 55 and 61 triplet repeats. In the present study, we did not detect the SCA12 mutation in 96 subjects with dominant cerebellar ataxia, which includes a diverse, unselected representation of ethnic groups. Similarly, in a recent report, no expansions were found in 392 unrelated patients from the United Kingdom, including 99 with a dominant family history. Furthermore, no expanded SCA12 alleles were detected in recessive or sporadic cases of ataxia in the present study or in previous reports. The unusual clinical presentation in affected members of family R and the rarity of CAG repeat expansion at the SCA12 locus among patients with ataxia suggest that SCA12 should be considered in cases with atypical phenotype, especially if limb tremor is present in the initial clinical picture. However, expansions at the SCA12 locus are rare in more typical dominant ataxias and account for very few cases of spinocerebellar degeneration.

The SCA12 allele distribution and heterozygosity index in our patients were similar to those previously described. A (CAG)10 repeat was the most frequently detected allele among patients with ataxia in the present study. The largest allele found had 22 repeats, which is
within the proposed nonpathogenic range suggested by other reports. Additional studies of patients with expansions are required to better define the pathogenic range of SCA12 and to investigate whether a correlation exists between repeat size and severity of the disease.

Recent functional genomic studies have shown that the SCA12 mutation affects the expression of a downstream reporter gene in vitro, suggesting that the CAG expansion near the 5' end of the PPP2R2B gene is pathogenic and not a benign polymorphism in linkage disequilibrium with a causal mutation. However, the molecular and cellular effects of the SCA12 mutation remain to be characterized.

SCA12 and some of the other mutations recently associated with ataxia are rare, suggesting that a number of as yet unidentified genetic defects underlie spinocerebellar degeneration of unknown origin.

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REFERENCES


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