Molecular Genetics of Hereditary Spinocerebellar Ataxia

Mutation Analysis of Spinocerebellar Ataxia Genes and CAG/CTG Repeat Expansion Detection in 225 Italian Families

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Background: Autosomal dominant cerebellar ataxias are a clinical and genetically heterogeneous group of progressive neurodegenerative diseases, at present associated with 22 loci (spinocerebellar ataxia [SCA] 1-SCA8, SCA10-SCA19, SCA21, SCA22, fibroblast growth factor 14 [FGF14]-SCA, and dentatorubral-pallidolysian atrophy [DRPLA]). The relevant gene has been identified in 12 cases (SCA1-3, SCA6-8, SCA10, SCA12, SCA14, SCA17, FGF14, and DRPLA), and in all but the recently identified PRKCG and FGF14 genes, the defect consists of the expansion of a short nucleotide repeat.

Objectives: To investigate the relative prevalence of SCA1-3, SCA6-8, SCA10, SCA12, and SCA17 gene expansions in Italian families with hereditary ataxia, specifically to verify the occurrence of SCA10, SCA12, and SCA17 in Italy; and to analyze samples from probands specifically to verify the occurrence of SCA10, SCA12, and SCA17 to be very rare (approximately 1% each), and no case of SCA10 or SCA12 was identified. Half of the index cases (113/225) were negative for expansions in the known SCA genes. Repeat expansion detection analysis performed on 111 of these cases showed a CAG/CTG repeat expansion of at least 50 triplets in 22 (20%). Twenty-one of 22 expansions could be attributed to length variation at 2 polymorphic loci (expanded repeat domain CAG/CTG1 [CTG18.1], and only a few SCA8 (2/225) and SCA17 (2/225) families were detected. In patients negative for defects in known SCA genes, repeat expansion detection data strongly suggest that, at least in our population, CAG/CTG expansions in novel loci are an unlikely cause of the SCA phenotype.

Conclusions: The distribution of SCA1-3 and SCA6-7 gene mutations is peculiar in Italy. We found a relatively high frequency of SCA1 and SCA2 gene expansions; SCA3, SCA6, and SCA7 mutations were rare, compared with other European countries. No SCA10 or SCA12 and only a few SCA8 (2/225) and SCA17 (2/225) families were detected. In patients negative for defects in known SCA genes, repeat expansion detection data strongly suggest that, at least in our population, CAG/CTG expansions in novel genes should be considered an unlikely cause of the SCA phenotype.
are translated into polyglutamines, or by expanded trinucleotide/pentanucleotide repeats occurring in the promoter (CAG in SCA12), in the intron (ATTCT in SCA10), or in a noncoding RNA (CTG in SCA8).

The relative frequencies of SCAs vary significantly among different populations. This variability can be partly ascribed to founder effects, as shown in Portuguese-Brazilian or Japanese populations for SCA3, Scandianvians for SCA7, and Mexicans for SCA10. In most countries, mutations in the known SCA genes account for up to 40% to 60% of SCA index cases with 1 or more affected relatives, with the exception of a few inbred populations, as in southern Brazil, where this figure is close to 100%. It is reasonable to assume that at least a subset of the gene-orphan SCA families could harbor unidentified genes with CAG/CTG expansions in coding or noncoding regions. We conducted this study with the following aims: first, to investigate the relative prevalence of known SCA1-7 gene expansions in a group of 225 Italian families with hereditary ataxia, with the specific interest of verifying the occurrence of SCA10, SCA12, and SCA17, which has not been systematically investigated in Italy; and second, to use the repeat expansion detection (RED) technique, a protocol successfully used to identify new loci with CAG/CTG pathologic expansions in ataxias and other neurological syndromes, to examine patients with negative findings for mutations in the known SCA genes.

METHODS

PATIENTS

Two hundred twenty-six unrelated index cases were first selected from a large cohort of 1490 patients with a diagnosis of ataxia who were referred to our center from throughout Italy. Criteria for selection included (1) a progressive clinical phenotype in which ataxia was the prominent symptom and (2) a positive familial history, ie, at least 1 first-degree relative with ataxia. One hundred eighty-three families showed an autosomal dominant segregation (eg, 2 affected siblings with young healthy parents, or 1 parent who died at a young age). In the latter group, 1 patient was found to carry the GAA expansion in the Friedreich ataxia gene (FRDA1). Thus, this study was performed in 225 ataxic index cases. In 189 cases, the mean ± SD age at onset of the first neurological symptoms related to ataxia could be obtained (39 ± 16 years; range, 3-76 years). Clinical and genetic examination was performed with the informed consent of the subjects.

RESULTS

Partial clinical and genetic characteristics of some of the patients described herein have been previously reported, but molecular analysis was confined to the SCA1-7 loci. In the present study, 152 new index cases were investigated for SCA1-7 gene expansions, whereas previously reported cases underwent evaluation for SCA8, SCA10, SCA12, and SCA17 gene mutations.

IDENTIFICATION OF SCA EXPANSIONS AND SCREENING FOR THE ERDA1 AND CTG18.1 LOCI

Polymerase chain reaction (PCR) analysis of the SCA1-3, SCA6-8, and DRPLA gene expansions was performed as described. The size of SCA1-7 intermediate and fully pathologic alleles was confirmed by direct sequencing of PCR products. To rule out the presence of a very large expansion at the SCA8 locus, samples that appeared to be homozygous for a normal allele also underwent testing by means of Southern blot analysis. The repeat-containing regions of the SCA10, SCA12, and SCA17 genes and the polymorphic ERDA1, also known as D17S313, were amplified using appropriate primers (Table 1; PCR conditions are available on request from the corresponding author).

All fluorescent PCR products were run on an ABI Prism 377 automatic sequencer (Applera, Foster City, Calif), and the data were elaborated using the GeneScan 3.1 software (Applera). For SCA8, SCA12, and SCA17, the normal allele range was estimated in a group of 127 Italian healthy control subjects.

MICROSATELLITE ANALYSIS, CLONING, AND SEQUENCING

We performed SCA8 haplotype analysis using the following 4 microsatellites spanning an approximately 300-kilobase region centered on the SCA8 gene: D13S318, D13S1296, JSCA8-9, and D13S135. The primer sequences and PCR protocols, except for the JJSCA8-9 marker, were obtained from the Genome Database (available at: http://www.gdb.org).

To establish the size and sequence structure of SCA8 and SCA17 expanded alleles, amplified products were subcloned and sequenced. At least 4 to 8 independent clones for each expanded allele were analyzed.

REPEAT EXPANSION DETECTION

The RED technique was performed following published protocols with minor changes. Hybridization was performed using a (CAG)12 probe 3′-tailed with digoxigenin–uridine triphosphate using the DIG oligonucleotide tailing kit and revealed using the DIG chemoluminescent detection kit (Roche Diagnostics, Milan, Italy). For each test, a plasmid containing a (CAG)n insert was used as an internal positive control.

RELATIVE FREQUENCY OF SCAs IN ITALY

Consistent with previous observations, most families carried mutations in the SCA1 (48/225) or SCA2 (53/225) genes with similar frequencies (21% and 24%, respectively) (Table 2). Expansions in the SCA3, SCA6, SCA7, SCA8, and SCA17 genes appeared to be very rare (<1%), accounting for 4% of the families in our series. No patient had positive findings for expansions at the SCA10 or SCA12 loci. Overall, the mutation detection rate...
This mutation is still unclear. In the present series, we examined a 73-year-old woman in whom a progressive cerebellar syndrome manifested at 70 years of age. At the time of our examination, she had dysphagia and a mild neuropsychological deficit. Her MRI showed marked atrophy of the cerebellum and brainstem. No information was available about the parents, and the remaining members of the family could not be directly examined. Reported clinical data showed that 5 other sisters and 1 brother were also affected, presenting with a similar cerebellar syndrome. Both affected male members of the family had severe mental impairment and onset of age.

SCA8 FAMILIES

Since very few families have been described in which the disease unequivocally segregated with an expansion in the SCA8 gene, the clinical phenotype associated with this mutation is still unclear. In the present series, we identified 2 families apparently associated with an SCA8 expansion. In family SCA8-MI-1 (Figure 1), the proband (II:2) was a 79-year-old man who presented at 40 years of age with a progressive cerebellar syndrome, characterized by upper and lower limb ataxia, dysarthria, nystagmus, and pyramidal tract involvement. At the time of our examination, the patient was wheelchair bound and exhibited severe mental deterioration. Magnetic resonance imaging (MRI) showed severe cerebellar atrophy. His sister (II:3) was a 73-year-old woman in whom a progressive cerebellar syndrome manifested at 70 years of age. The 19-CAG allele is pathologic only when present on both alleles (Mariotti et al30).

Table 2. Expansions of Short Nucleotide Repeats Among 225 Italian Families With Hereditary SCA

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of Index Casesa</th>
<th>Relative Frequency vs Total, %</th>
<th>Relative Frequency vs ADCA, %</th>
<th>No. of Repeats in Tested Patients</th>
<th>Reference Values†</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCA1</td>
<td>48 (1)</td>
<td>21.3</td>
<td>25.3</td>
<td>37 to 60†</td>
<td>6 to 44§</td>
</tr>
<tr>
<td>SCA2</td>
<td>53 (1)</td>
<td>25.8</td>
<td>27.9</td>
<td>35 to 54</td>
<td>14 to 31</td>
</tr>
<tr>
<td>SCA3</td>
<td>2 (2)</td>
<td>&lt;1</td>
<td>1</td>
<td>73 to 86</td>
<td>13 to 47</td>
</tr>
<tr>
<td>SCA6</td>
<td>2 (2)</td>
<td>&lt;1</td>
<td>1</td>
<td>19 to 26</td>
<td>4 to 18</td>
</tr>
<tr>
<td>SCA7</td>
<td>2 (1)</td>
<td>&lt;1</td>
<td>1</td>
<td>43 to 70</td>
<td>7 to 35</td>
</tr>
<tr>
<td>SCA8</td>
<td>2 (1)</td>
<td>&lt;1</td>
<td>1</td>
<td>116 to 152</td>
<td>15 to 50§</td>
</tr>
<tr>
<td>SCA10</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>10 to 22¶</td>
</tr>
<tr>
<td>SCA12</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>7 to 31</td>
</tr>
<tr>
<td>SCA17</td>
<td>2 (1)</td>
<td>&lt;1</td>
<td>1</td>
<td>45</td>
<td>25 to 42¶</td>
</tr>
<tr>
<td>DRPLA</td>
<td>1 (1)</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>63</td>
<td>3 to 36</td>
</tr>
</tbody>
</table>

ADCA families assigned to known genes 112 49.8 58.9
ADCA orphan-gene families 78 34.7 41.0
Non-ADCA orphan-gene families 35 15.6
Total ADCA families 190 84.4

Abbreviations: ADCA, autosomal dominant cerebellar ataxias; DRPLA, dentatorubral-pallidoluysian atrophy; SCA, spinocerebellar ataxia.

*aNumbers in parentheses represent index cases classified as compatible with dominant and recessive inheritance.
†Available at: http://www.geneclinics.org.
‡Includes 1 case with 37 uninterrupted CAG triplets, whose 2 first-degree cousins carried a pure (CAG)41.
§Alleles 39-40 are pathologic only if uninterrupted.
¶These values include our control subjects.
||The 19-CAG allele is pathologic only when present on both alleles (Mariotti et al30).
†‡Available at: http://www.neuro.wustl.edu/neuromuscular/ataxia.©2004 American Medical Association. All rights reserved.

Figure 1. Pedigrees and genotyping from 2 families with expansion of the gene for spinocerebellar ataxia 8 (SCA8). Haplotypes of the 4 microsatellite markers spanning approximately 300 kilobases centered to the SCA8 locus are shown. Markers are positioned according to the centromeric (Cen) and telomeric (Tel) orientation. The number of CTA and CTA repeats within each SCA8 expanded allele is indicated in brackets. Arrows indicate probands; squares, male members; circles, female members; diamond, member of unknown sex; dagger, years of age at death; open shapes, healthy members; question marks, undetermined disease status; solid shapes, symptomatic patients; and slashes, deceased.
neurological signs in the fourth decade of life. By contrast, the disease manifested in the affected female members at an older age, and severe cognitive impairment did not develop. All 8 affected siblings had type 2 diabetes mellitus. Molecular analysis could be performed on patients II:2 and II:3 only. In both siblings, expansions ranging from 114 to 152 CTG + CTA triplets were present on both alleles with the same haplotype (Figure 1), suggesting a possible consanguinity.

In the second family (SCA8-MI-2; Figure 1), the proband (II:1) presented at 51 years of age with progressive gait ataxia, dysarthria, and type 2 diabetes mellitus. A mild motor-sensory polyneuropathy later developed. A computed tomographic scan showed marked atrophy of the cerebellum and brainstem with enlargement of peripontine spaces, which prompted a diagnosis of olivopontocerebellar atrophy. The disease was slowly progressive, and the patient was still able to walk 2 years before death, which occurred at 74 years of age. His mother and sister were reported to be affected with similar clinical features, with a disease onset at 55 and 41 years of age, respectively. The proband, the only subject in this family available for SCA8 genetic testing, showed 139 CTG + CTA triplets ([CTG]128+[CTA]2).

**SCA17 FAMILIES**

This study has also detected 2 families with SCA17 expansions (SCA17-MI-1 and SCA17-MI-2, Figure 2). In family SCA17-MI-1, the proband (II:2) was a 42-year-old man who had manifested a rapidly progressive form of severe cerebellar ataxia since 41 years of age. Recently, he showed signs of mild mental impairment. His sister, aged 47 years, showed similar features of ataxia with onset at 38 years of age. However, the cerebellar symptoms appeared to be more severe, and the picture was complicated by the presence of chorea and severe dementia. In both siblings, MRI showed marked cerebellar atrophy and, in the brother, the enlargement of the peripontine spaces. Both parents appeared to be healthy at older than 60 years. In family SCA17-MI-2, 6 members in 3 generations appeared to be affected by a progressive cerebellar syndrome with gait ataxia and dysarthria. The ages at onset ranged from 25 to 35 years. Three individuals (I:2, II:2, and II:3) had a clear mental deterioration, whereas the proband (III:5) and his sister (III:2) exhibited very mild signs of cognitive impairment. Their younger first-degree cousin (III:6) had normal intellectual performance results, suggesting that occurrence of cognitive disturbances might depend on disease duration. The other affected relatives presented with a similar disease course characterized by gait and limb ataxia, severe dystarthishia, and dementia.

The probands of both families carried a 45-repeat SCA17 allele exhibiting a similar CAG + CAA alternate structure, with the expansion confined to the last CAG stretch ([CAG]25–26) (Figure 2). These expanded (CAG + CAA)45 alleles are smaller than the generally reported pathologic threshold of 46 repeats (reference range, 29–42 glutamines; Table 2). However, the lower limit for SCA17 pathogenic expansions is still controversial, since a (CAG/CAA)45 expansion in the SCA17 gene has been recently described in a patient with mental deterioration and ataxia.

**RED ANALYSIS**

Overall, 113 (50.2%) of our 225 cases of familial SCA do not involve known autosomal dominant cerebellar ataxia genes. Even if only families with a likely dominant transmission are considered, the proportion of genetically undefined families remains high (78/190 [41.0%]; Table 2). Since a large variety of genes containing CAG/CTG repeats exist in the human genome, we searched for expanded CAG/CTG repeats in 111 of the 113 gene-orphan SCA families by means of the RED technique, which can detect triplet repeat expansions without prior knowledge of their chromosomal location. Most of the healthy subjects (approximately 70%) show ligation products of 20 to 40 CAG/CTG repeats (i.e., false-positive signals), derived from polymorphic loci of no pathologic relevance. Therefore, only expansions of at least 50 repeats were considered significant. As shown in Table 3, significant expansions were found in 22 (19.8%) of 111 gene-orphan SCA families; all 22 probands were further typed at 2 polymorphic loci (CTG18.1 and ERDA1). Known to harbor alleles with large CAG/CTG repeats, the results of RED analysis and the genotypes of the latter polymorphic loci are summarized in Table 3. Indeed, the expansion was likely due to ERDA1 (19/22 probands [86%]) or to CTG18.1 (2/22 [9%]) (Table 3). In only 1 proband, a 46-year-old woman who showed a progressive cerebellar syndrome associated with cognitive impairment and choreoathetosis at 35 years of age, a 60-repeat expansion could not be ascribed to either polymorphism. She had a sister with a similar phenotype and 2 sons with a diagnosis of cryptogenic focal epilepsy.
prompted us to perform a genetic test for DRPLA, which disclosed the presence of an expanded allele of 63 CAG repeats. Genotyping at the DRPLA locus in all remaining SCA gene-orphan patients did not reveal any further positive cases.

Table 3. RED Analysis of 111 Familial Ataxic Patients With Negative Findings for Expansions at Known SCA Gene

<table>
<thead>
<tr>
<th>No. of CAG/CTG Triplets</th>
<th>No. of Index Cases</th>
<th>ERDA1</th>
<th>CTG18.1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Index Cases</td>
<td>No. of Cases</td>
<td>No. of Repeats</td>
</tr>
<tr>
<td></td>
<td>ADCA</td>
<td>Non-ADCA</td>
<td>ND</td>
</tr>
<tr>
<td>≤40</td>
<td>59</td>
<td>30</td>
<td>ND</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>60</td>
<td>2</td>
<td>3*</td>
<td>3</td>
</tr>
<tr>
<td>70</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>80</td>
<td>1</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>35</td>
<td>19</td>
</tr>
</tbody>
</table>

Abbreviations: ADCA, autosomal dominant cerebellar ataxias; ERDA1, expanded repeat domain CAG/CTG 1; CTG18.1, CTG repeat at 18q21.1; ND, not determined; RED, repeat expansion detection technique; SCA, spinocerebellar ataxia.
*Includes 1 patient with an expansion in the gene for dentatorubral-pallidoluysian atrophy.
†Includes 1 patient with 59 and 70 repeats at the ERDA1 locus.

SCAs in Italy

As suggested in preliminary studies,25,29 the distribution of SCA gene mutations in Italy appears to be peculiar. First, in addition to the relatively high frequency of SCA1 and SCA2 expansions, compared with other European countries, SCA3 expansions appear to be very rare: only 2 (0.9%) of 225 index cases. This represents a unique feature of the Italian population with SCA, because SCA3 is quite frequent in many countries with different ethnic backgrounds, such as Portugal (approximately 80%), Germany (approximately 40%), Japan (approximately 40%), and France (approximately 30%).18 It is also remarkable that both SCA3 families originated from Liguria, a region on the northwestern coast of Italy, suggesting a possible common ancestor. Second, the SCA6 mutation also appeared to be very rare (2/225), with a relative frequency (0.9%) similar to that observed in France but significantly different from that found in Japan, Germany, the United States, and Australia (10%-20%).19 Finally, the SCA7 mutation was also very rare (2/225), as observed in all other populations except Scandinavia.16

No SCA10 and SCA12 gene mutations were detected. Indeed, the SCA10 expansion appears to be restricted to a few families of Mexican origin only, probably owing to a founder effect.5,17 The SCA12 mutation so far has been observed in only 2 families of German and Indian origin.4,6 These findings indicate that genetic testing for SCA10 and SCA12 should be considered in the presence of specific signs only, such as ataxia and seizures (SCA10) or ataxia and initial tremor (SCA12).

SCA8 Families

The clinical features of our patients with SCA8 were partially similar to those reported so far, and were characterized by an adult-onset, extremely slow progressive cerebellar ataxia.31,33 In the 2 affected male members from family SCA8-MI-1, the disease included a late complication of a severe mental deterioration, a feature previously reported by Juvonen et al31 in 40% of Finnish patients with SCA8.

Altogether, available data indicate that a clinical hallmark cannot be clearly assigned to this disease.31,33 In both families, the expansion sizes were within or exceeded the putative pathologic range of 110 to 130 repeats reported by Koob et al.3 The penetrance and expressivity of expanded SCA8 alleles is a complex issue, and the actual pathogenic role is still debated.33 First, repeats beyond the putatively normal upper limit have been observed in healthy subjects.31,34 Second, expanded CTG repeats have been found to occur with similar frequencies in patients with ataxia and those with a mixture of psychiatric diagnoses.31,33 However, available evidence also indicates that (CTG)n alleles in the range of 90 to 130 repeats appear to be more frequently associated with ataxia compared with controls.3,33 Altogether, these observations imply that the SCA8 (CTG)n expansion is a low-penetrance disease-causing mutation or a polymorphism genetically linked to a different nearby disease gene.31,33

SCA17 Families

The clinical features of our SCA17-affected individuals were similar to those described so far. The phenotype was dominated by a mild-to-severe mental deterioration, with cerebellar ataxia variably associated with movement disorder. Initially, some of the patients had received a diagnosis of olivopontocerebellar (multisystem) atrophy (family SCA17-MI-2) or prion disease (family SCA17-MI-1). As previously observed, no clear correlation could be established between the size of the expansion and the age at onset.

RED Analysis

The implications of our findings are 2-fold. First, they confirm the efficacy of RED to detect trinucleotide expansions of pathologic relevance. Second, they indicate that DRPLA analysis should definitely be included in the diagnostic protocol for hereditary ataxias, even in the absence of a suggestive clinical phenotype or a clear dominant transmission pattern.
Overall, our results strongly suggest that, at least in our series of patients, the association of expanded CAG/CTG repeats with novel SCA loci is quite unlikely. These data are in agreement with the reported lack of polyglutamine-containing novel proteins in patients with autosomal dominant cerebellar ataxias and negative findings for known SCA loci, resulting from a screening with the 1C2 monoclonal antibody. However, a series of circumstances might hamper the efficiency of RED in detecting pathogenic CAG/CTG expansions. First, large CAG/CTG repeats at ERDA1 and/or CTG18.1 polymorphisms may hide a true pathogenic expansion. Should this be the case, we would expect that only a few of our 21 RED-positive cases could be ascribed to such a condition, as the allelic frequencies at the latter loci in our series of patients were similar to those of healthy subjects. Second, a pathogenic expansion of no greater than 40 repeats, as in SCA6 (19-33 repeats), would not become evident at RED analysis. Third, short CAG expansions intercalated with a few CAA repeats, the alternative codon for glutamine, cannot be detected by means of RED, although they may result in a pathologic polyglutamine stretch, as in SCA17 (Figure 2). Finally, oligonucleotide repeats different from CAG/CTG could be involved in SCA, as shown by the pentamer expansion in SCA10.

Although the SCA10 mutation is the only example of a nontrinucleotide repeat so far associated with hereditary ataxias, it is conceivable that a variety of other short nucleotide expansions will be found as a cause of SCA, because repeat expansions remain the most likely molecular basis for the anticipation phenomenon. Indeed, in our series of 113 gene-orphan SCA families, anticipation was clearly present in 14 (12%), a figure likely underestimated owing to the general lack of clinical de-


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