Genetic and Clinical Analysis of Spinocerebellar Ataxia Type 8 Repeat Expansion in Italy

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**Background:** The spinocerebellar ataxias (SCAs) are clinically heterogeneous disorders caused by triplet repeat expansions in the sequence of specific disease genes. Spinocerebellar ataxia type 8 (SCA8), originally described in a family characterized by pure cerebellar ataxia with slow disease progression, presents with expansion of combined CTA/CTG repeats.

**Objective:** To perform SCA8 repeat expansion analysis in a heterogeneous group of ataxic patients, to determine the prevalence of this mutation in our patients and establish the frequency of expanded CTA/CTG repeats in a large group of control subjects.

**Patients:** One hundred sixty-seven patients affected by sporadic, autosomal dominant and recessive hereditary ataxia were clinically examined and analyzed for SCA8 expansion. We further studied 161 control subjects and 125 patients with psychiatric disorders.

**Results:** We found abnormally expanded CTA/CTG repeats in 5 ataxic patients, 3 of them characterized by pure cerebellar ataxia. One patient had vitamin E deficiency and 1 patient with a sporadic case was affected by gluten ataxia. No evidence of expanded alleles was found in healthy control subjects and in patients with psychiatric disorders.

**Conclusions:** Our data support the evidence that CTG expansions may be linked to SCA8, since the pathogenic expansions have been found only among patients with genetically unidentified forms of hereditary and sporadic ataxia. Patients carrying expanded alleles present peculiar phenotypic features, thus suggesting that unknown additional factors could probably predispose to the disease.

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**S**pinocerebellar ataxia type 8 (SCA8) is the first form of ataxia caused by a CTG triplet repeat expansion. This disorder, originally described in a family characterized by pure cerebellar ataxia with slow disease progression, shows clinical features similar to those of other spinocerebellar ataxias (SCAs), including limb and truncal ataxia, dysarthria, and nystagmus. The SCA8 has been distinguished among triplet disease disorders both in terms of the transcribed repeat motif (CTG) and for its location in the noncoding region of the gene, resulting in an untranslated expansion at the 3′ end of RNA. The existence of an antisense transcript, encoding a novel actin-binding protein (KLHL1), has been recently reported, suggesting that the pathogenic effect of SCA8 expansion may result in an alteration of KLHL1 messenger RNA stability or processing. However, the role of CTG expansion in the pathogenesis of SCA8 and the molecular mechanism responsible for the disease remain to be clarified.

The CTG repeat tracts on expanded alleles are often interrupted by other triplet motifs (CCG, CTC, CCA, and CTT, with a more frequent polymorphic CTAn). These interruptions within the CTG repeat tract might potentially stabilize such expansions, influencing the penetrance of expanded SCA8 alleles.

Normal alleles have a repeat size varying from 16 to 91 combined CTG/CTA repeats, whereas patients with SCA8 carry expanded alleles with 107 to 127 pure CTGs. In addition, pathogenic CTG expansions, resulting from maternal inheritance, seem to represent a peculiar feature of the disease, in contrast to the majority of dominant SCAs showing paternal transmission. The evidence that expanded CTG repeats undergo significant length changes during intergenerational transmission may explain the reduced penetrance of SCA8 ataxia.

Possible pathogenic combined repeats, ranging from 92 to 250 CTA/CTG,
GENETIC ANALYSIS

Genomic DNA was extracted from peripheral blood lymphocytes. Polymerase chain reaction amplification was carried out with slight modifications of the published protocol,1 with the use of the following primers: SCA8-F3: 5’-TTTGAGAAAGGCTTGTGAGGACTGAGAATG–3’; and SCA8-R4: 5’-GGTCCTTCTATTTAGAAAACCTGCT–3’, where F indicates forward; R, reverse.

Amplification was done in a DNA thermal cycler (Model 9600; Perkin-Elmer, Norwalk, Conn) with 35 cycles of amplification performed at 94°C for 35 seconds, 64°C for 45 seconds, and 72°C for 45 seconds.

Ten microliters of each amplified DNA sample was resolved on 3% agarose gel, stained with ethidium bromide, and visualized by UV light. Size range of normal and expanded alleles was determined for comparison with an appropriate DNA molecular weight standard. For an accurate estimation of the repeat size, the polymerase chain reaction products of all suspected cases of SCA8 were analyzed with an automated DNA sequencer (ALF Express; Pharmacia LKB, Uppsala, Sweden). Data were processed with fragment analysis software (Fragment Manager; Pharmacia) with the use of a size marker (Size 50-500; Pharmacia) according to the manufacturer’s protocol.

Except for 30 patients with a known genetic defect (6 patients with SCA1, 22 patients with SCA2, and 2 patients with SCA3), all the subjects with ataxia were negative for CAG triplet repeat expansions specific for the other spinocerebellar ataxia (SCA1, SCA2, SCA3/Machado-Joseph disease, SCA6, SCA7, SCA8, and SCA12) types. Moreover, all the 22 patients with FA examined present a GAA specific expansion in the FRDA gene.

We analyzed 167 patients with ataxia (mean±SD age, 48.8±19.4 years), including 77 patients with autosomal dominant cerebellar ataxia, 25 subjects with autosomal recessive cerebellar ataxia, and 65 patients with sporadic cases, 56 of whom had idiopathic late-onset cerebellar ataxia. Clinical diagnosis was made according to the criteria proposed by Harding.1 In particular, idiopathic late-onset cerebellar ataxia was defined by the presence of progressive cerebellar ataxia without evidence of a focal or nonfocal symptomatic origin of the disease and by the absence of neurodegenerative disorder in relatives without evidence of consanguinity of parents. Among patients with hereditary ataxia, 30 were carrying a CAG pathogenic expansion in one of the known ataxia loci6 (6 patients with SCA1, 22 patients with SCA2, and 2 patients with SCA3) and 22 patients with Friedreich ataxia (FA) were bearing a pathogenic GAA expansion in the FRDA gene.

As control subjects, we examined 161 healthy individuals aged 19 to 106 years (mean age, 74.5±25.1 years).

Since previous results7 have shown pathogenic CTG expansion in psychotic diseases not characterized by ataxia, we also genotyped 64 patients with schizophrenia (mean±SD age, 47.5±14.6 years) and 61 patients (mean±SD age, 54.1±15.9) with a familial history of bipolar affective disorders. Patients and control subjects participating in this study originated from Italy. Informed consent was obtained for all of the genetic analyses after the nature of the procedure had been fully explained.

have been reported in ataxic patients since the original report.1 In addition, recent collaborative studies,8-10 using large panels of patients and control subjects, reported nonspecific SCA8 pathogenic expansions in healthy subjects and in patients with different neurologic and psychiatric disorders, thus suggesting that CTG expansions linked to SCA8 may represent rare polymorphisms. We have analyzed SCA8 CTA/CTG expansions in a heterogeneous group of ataxic patients to better define the molecular features of these peculiar repeats and to individuate a common clinical phenotype characterizing this new form of ataxia. Moreover, we have investigated the prevalence of SCA8 expansion through triplet analysis of a pool of psychiatric (bipolar and schizophrenic) patients and control individuals.

RESULTS

We analyzed the distribution of the SCA8 expansion in 167 patients clinically affected by ataxia and found 8 subjects (5 patients with ataxia and 3 apparently unaffected relatives) with abnormally expanded CTA/CTG repeats. No family history of dominant ataxia was present in any of the patients carrying the SCA8 expansion.

In particular, pathogenic SCA8 repeat expansions were identified in 3 patients (belonging to families DL-1 and DS-1; Figure 1) with an apparent autosomal recessive pure cerebellar ataxia (characterized by affected siblings and unaffected parents), in 1 patient with ataxia who had vitamin E deficiency (family PN-1), and in 1 patient with a sporadic case (PR-1) with gluten ataxia. An expanded allele also has been found in 2 unaffected parents and in 1 unaffected brother.

The length of the repeat tracts among individuals carrying at least 1 expanded SCA8 allele ranged between 90 and 320 combined CTA/CTG (Figure 2).

Repeats in the normal range were observed in the remaining examined subjects (906 total chromosomes, including 292 patients and 161 controls). In particular, all of the studied subjects not carrying SCA8 expansion in any allele had 15 to 75 triplets (Figure 2). The most strongly represented allele contained 25 repeats, accounting for 32.9% of all normal analyzed chromosomes. The distribution of nonexpanded alleles (n=906) did not differ from the size range previously reported.1,10

Family PN-1 (Figure 1) exhibited genetic maternal transmission (+215 repeats) from the healthy mother (presenting 105 CTA/CTG repeats, close to the pathogenic range). The proband was 30 years old and had had a clinical picture resembling FA without cardiac abnormalities since the age of 7 years. Hyperreflexia and gait disturbances were detected. She had become wheelchair bound by age 17 years. Genetic testing showed no specific FA expansion (9 GAA triplets in both FRDA alleles).
Recently, low serum vitamin E concentration (0.3 µg/mL; normal range, 5.0-15.2 µg/mL) was detected. The patient in family PR-1 with sporadic disease developed early symptoms at 25 years age, when he noticed tremor in his arms while writing but not while at rest. He successively developed unsteadiness, hyperreflexia, and dys tonic postures. Pure cerebellar atrophy with predominant involvement of the cerebellar vermis and of both hemispheres was demonstrated by brain magnetic resonance imaging. No electrophysiologic abnormalities were detected. Clinical diagnosis of sporadic cerebellar ataxia was made. Since he occasionally had diarrhea, an endoscopic biopsy of the distal duodenum was performed, disclosing subtotal villous atrophy, crypts, hyperplasia, and moderate increase in intraepithelial lymphocyte cell count. Positivity for IgA antiendomysial antibodies and anti–tissue transglutaminase confirmed the diagnosis of gluten ataxia. Genetic analysis showed a heterozygous combined 159-CTA/CTG expansion at the SCA8 locus, while his parents had both alleles in the normal range (22/24 repeats). Genetic analysis excluded patient PR-1 from CAG triplet expansions in the other identified SCAs and from pathogenic GAA repeats in the frataxin gene.

Two affected brothers, belonging to family DS-1, had pure cerebellar ataxia. Both carried an expanded allele of 90 CTG repeats. Ages at onset was 35 and 40 years, with symptoms of gait ataxia, unsteadiness, dysarthria, slow progression of the disease, and a brain magnetic resonance imaging appearance of hemispheric and vermian cerebellar atrophy. One patient showed mild pyramidal signs with hyperreflexia and bilateral Babinski sign. The asymptomatic father had a larger expansion of 105 CTG repeats, demonstrating a contraction of 15 repeats during paternal transmission. Brain magnetic resonance imaging in the father showed a diffuse cortical and cerebellar atrophy.

The patients in family DS-1 had normal results for IgA and IgG antigliadin antibodies and for vitamin E serum levels. The asymptomatic brother in family DS-1 and the mother in family PN-1 were personally examined by us and were free of any clinical neurologic signs. One patient with sporadic late-onset cerebellar ataxia (family DL-1) also showed a pathogenic expansion of 110 SCA8 repeats. His parents were dead at the time of our study. His brother had a similar history of tremor and probable cerebellar ataxia, but he lived in a different country and was not examined.

We investigated the occurrence of the SCA8 triplet expansion in a large Italian sample including patients with...
ataxia (65 sporadic, 77 autosomal dominant cerebellar ataxia, 3 autosomal recessive cerebellar ataxia, 22 FA), patients with different psychiatric disorders (64 schizophrenic, 61 bipolar), and 161 control subjects. We found 8 subjects (5 patients with ataxia and 3 apparently unaffected relatives) with abnormally expanded CTA/CTG repeats. No pathogenic expansion has been detected in the other subgroups of patients and in the sample of control subjects examined (453 total subjects).

In contrast with previous reports, suggesting that SCA8 expanded CTG repeats could be a nonpathogenic polymorphism in linkage disequilibrium with an ataxia locus, our results support the evidence that SCA8 expansions are rare (3% of all ataxic patients) but may confer a susceptibility predisposing to the ataxic phenotype. These data are confirmed by the virtual absence of pathogenic allele expansions on nonataxic patients' chromosomes (0/572 in our study) and by the low frequency (4.3%=5/115) of expansions among patients with genetically unidentified forms of hereditary (50 patients) and sporadic (65 patients) ataxia. In addition, the absence of this expansion among ataxic patients in whom a known genetic defect has already been diagnosed (30 patients carrying pathogenic CAG repeat expansions in SCA1, SCA2, SCA3, and 22 patients with FA with GAA expansion in the FRDA locus) could argue against the fact that CTA/CTG triplet expansion represents a nonspecific genetic factor associated with ataxia.

Nonpenetrant expansions found both within families with ataxia and, rarely, in the general population have provided controversial results. Large trinucleotide (CTA/CTG) repeat alleles have been observed in 1.25% of patients with various psychiatric disorders, but none affected by, or with a family history of, spinocerebellar ataxia. Interestingly, our analysis, performed on 125 psychiatric patients, detected only 1 patient, with bipolar disorder, carrying a normal large allele of 75 CTA/CTG repeats.

Furthermore, recent evidence suggested that long CTG repeats (>250 repeats) in the SCA8 gene may not be pathogenic, possibly because they are not expressed or because they might alter RNA processing or stability.

The presence of a 320-triplet SCA8 expansion in 1 of our ataxic patients (in family PN-1) seems to contradict this hypothesis, suggesting a possible role also for large expansions in the occurrence of ataxia. Size distribution of our normal SCA8 chromosomes is similar to the reported trimodal distribution, describing classes of small (<21 CTGs); intermediate (22-33 CTGs), and large (40-75 CTGs) alleles. The analysis of nonexpanded SCA8 allele population provides evidence of a very low percentage (1%) of alleles predisposing to further expansion toward the pathogenic range during transmission.

The limited number of patients with SCA8 carrying pathogenic expansion has not allowed us to establish a correlation between repeat length and clinical disease phenotype. A maternal transmission in one family (PN-1) and a paternal contraction in family DS-1 have been detected (as reported by others in a 2-generation Japanese pedigree).

We also confirmed the marked heterogeneity of the SCA8 clinical picture. Unusual features such as vitamin E deficiency reported in family PN-1, or association (in family PR-1) with celiac disease (as previously reported in a patient carrying SCA8 expansion), suggest that the presence of other additional unknown factors (which were absent in their respective parents) may probably predispose to ataxia. In light of this hypothesis, CTA/CTG in the SCA8 locus could influence but sometimes was not sufficient to develop the ataxic phenotype.

A possible epistatic effect of the SCA8 gene on susceptibility to malabsorption disturbances leading to ataxia could also be conjectured. Computer-based homologic comparison with other genes linked to malabsorption gave no evidence of any positive result until now.

Additional investigations will be required to precisely determine the function and the molecular mechanisms through which these expansions could be involved in the pathogenesis of this rare disease.

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