Clinical Features and ATTCT Repeat Expansion in Spinocerebellar Ataxia Type 10

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Background: Spinocerebellar ataxia type 10, an autosomal dominant disease characterized by ataxia and seizures, is caused by a large expansion of an unstable ATTCT pentanucleotide repeat.

Objectives: To characterize the phenotypic expression of spinocerebellar ataxia type 10 and to examine the genotype-phenotype correlations in 2 large families.

Design: Clinical characterization and genotype-phenotype correlation.

Setting: Studies at 2 medical schools with private practice referral.

Patients: Twenty-two affected individuals from 2 large Mexican American pedigrees.

Results: Of the 22 individuals, ataxia was the initial symptom in 21; seizure disorders developed in 11, mostly within several years following the onset of ataxia. The seizure frequency was different in the 2 families: 3 (25%) of 12 had seizures in family 1, and 8 (80%) of 10 had seizures in family 2 \((P = .01)\). A brain magnetic resonance imaging or computed tomographic scan showed cerebellar atrophy in all patients examined. An electroencephalogram demonstrated epileptiform discharges in 4 of 8 patients studied. Although anticipation was apparent in both families, only family 1 showed a strong inverse correlation between age of onset and repeat number \((r^2 = 0.79, P = .001)\). In family 1, 8 transmissions, of which 7 were paternal, resulted in an average gain of 1940 repeats. In contrast, despite anticipation, 2 affected male subjects transmitted their expanded alleles to 8 progenies, with an average loss of 755 repeats, in family 2.

Conclusions: Seizure is an integral part of the spinocerebellar ataxia type 10 phenotype, with documented morbidity and mortality. Family-dependent factors may alter the frequency of the seizure phenotype and the pattern of intergenerational repeat size changes, making the genotype-phenotype correlation complex.

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The spinocerebellar ataxias (SCAs) are a genetically heterogeneous group of neurodegenerative disorders. Traditionally, the autosomal dominant cerebellar ataxias (ADCA) have been classified according to phenotype. In the classification proposed by Harding, the ADCA are divided into 3 different groups (ADCA I, II, and III) depending on the presence or absence of associated features, such as retinopathy or other extracerebellar signs. However, there are significant phenotypic overlaps and variability among these disorders, making a definitive clinical diagnosis difficult. Recent advances in the molecular genetics of these disorders have led to a genotypic classification using the SCA prefix. They are an expanding group of disorders, and 15 different loci (SCA1-8, SCA10-14, and SCA16-17) have been genetically mapped. In 6 of the disorders (SCA1-3, SCA6-7, and SCA17), the disease-producing mutation is an expansion involving the trinucleotide repeat CAG in the coding portion of the gene. In 2 of the disorders (SCA8 and SCA12), the reported mutations are in the noncoding portions of the disease genes. However, in SCA8, unlike SCA12, the expanded trinucleotide repeat involves a CTG rather than a CAG expansion.

The clinical and genetic analysis of a large Mexican American pedigree with an ADCA has been reported. Genetic analysis showed that in this and one other large family, the disease gene localized to chromosome 22. More recently, the molecular basis of SCA10 as an expansion mutation of a pentanucleotide (ATTCT) repeat in intron 9 of the SCA10 gene was established. Analysis of 604 chromosomes from unaffected individuals of various ethnic origins, including Mexicans, showed a range of 10 to 22 ATTCT repeats with no evidence of expansions, whereas affected individuals had expansions of greater than 800 ATTCT repeats.
PATIENTS AND METHODS

PATIENTS

The index patient (patient III:1 of family 1) (Figure 1A) was identified and examined at University of Southern California Medical Center. The clinical descriptions and results of investigations of a part of this pedigree were previously reported.9 The members of family 2 (Figure 1B) were identified through the index patient (III:6) and examined by one of us (M.A.) and then also examined at Baylor College of Medicine.11 All living affected members were examined, and the clinical status of deceased individuals was determined with a corroborating history from at least 2 independent sources.

MUTATION ANALYSIS

After obtaining informed consent, 40 mL of blood was obtained and genomic DNA was extracted by standard methods. Southern blot analysis was performed to determine the ATTCT repeat number, as previously described.12 Briefly, 10 µg of genomic DNA was digested with EcoRI, separated by gel electrophoresis (0.8% agarose), and transferred to a membrane (Hybond N+; Amersham Biosciences, Piscataway, NJ). An SCA10 probe was generated by polymerase chain reaction, amplifying an 800-base pair fragment located upstream to the pentanucleotide repeat from a genomic DNA clone of the region using the following primers: DanL (5'-TCCCTCTCGATTTCTGCG-3') and DanR (5'-TGGCATCTTTCTATTG-3').

This probe was radiolabeled with phosphorus 32 by random priming, and hybridization was performed in Church buffer (0.1mM EDTA, pH 8.0; 0.5M sodium phosphate, pH 7.2; and 7% sodium dodecyl sulfate) at 60°C overnight; the membrane was washed in 2X SSC (at 60°C for 5 minutes), 1X SSC (at 60°C for 20 minutes), and 0.5X SSC (at 60°C for 20 minutes) with 0.1% sodium dodecyl sulfate and then analyzed with autoradiography.

knowledge, this novel class of mutation is one of the largest microsatellite repeat expansions described in the human genome. The segregation of the expansion in affected individuals, the absence of the mutation in healthy control subjects, and the expression of SCA10 in the central nervous system provide compelling evidence that this is the disease-producing mutation. However, the mechanism of how the expanded allele causes SCA10 is unknown and will be the subject of future studies. We present the genotype-phenotype analysis of extended pedigrees of the original families, including new clinical data on recently identified members.

RESULTS

PHENOTYPE ANALYSES

Since the original report,9 changes in the clinical status of some of the individuals have occurred in family 1 (Table and Figure 1A). One of the subjects in family 1 (patient II:2) died of complications of status epilepticus at the age of 62 years. His cranial computed tomographic scan showed marked cerebellar atrophy with no lesions suggestive of cystercerosis. He had developed seizures at the age of 56 years and had been treated with carbamazepine and phenytoin; however, no serum levels were available for review. An electroencephalogram (EEG), which had been performed several weeks before death, confirmed that he had significant ongoing bilateral epileptiform discharges. No autopsy was performed. Another affected member in family 1 (patient III:2) developed a complex partial seizure disorder at the age of 50 years. The diagnosis was based on a reliable medical history, abnormal EEG results, and a good response to carbamazepine. Her cranial computed tomographic scan showed 1 small calcified lesion typical of inactive cystercerosis in the right occipital lobe. We identified an additional branch of the pedigree, in which 2 of 13 living members were symptomatic, including 1 (patient II:1 of family 1) who had a history of complex partial seizures with generalization and subsequently died of the complications of a fall secondary to the seizure disorder. Thus, of the living patients examined, 3 (25%) of 12 experienced seizures, either complex partial seizures or complex partial seizures with secondary generalization. If we include all affected individuals in this pedigree in whom a reliable medical history could be obtained, there are 4 (25%) of 16 affected individuals who experienced seizures.

Reexamination of 2 individuals in family 1 (patients III:1 and III:5) and an initial examination of 1 other affected individual in family 1 (patient IV:5) showed that there may be some mental status changes. Although a Spanish translation of a Mini-Mental State Examination showed normal scores with no major deficits in attention, memory, or language, there seemed to be...
changes in personality. All 3 had a flat affect and apathy, showing a general disinterest in their surroundings. All patients in family 1 denied any vegetative symptoms of depression. More detailed psychological studies will be performed to further investigate this clinical impression.

For family 2 (Table and Figure 1B), the original report described only outlines of the clinical phenotype. Since then, we identified 1 new affected member (patient III:0). However, 2 individuals (individuals 20 and 23 described in the article by Matsuura et al) who had subtle balance problems on the initial examination and scored as being affected in 1999 were excluded because of their uncertain phenotype and the absence of the SCA10 mutation in their DNA. Altogether, 10 patients had progressive cerebellar ataxia, including variable degrees of unstable gait and posture, scanning speech, dysmetria, intention tremor, dysdiadochokinesia, and fragmented ocular pursuit. Gait imbalance was the first symptom in all affected members. Most patients showed ocular dysmetria and gaze-evoked nystagmus of variable magnitude, sometimes causing visual disturbances; however, no patients showed ophthalmoparesis. Two patients in family 2 (patients II:1 and II:2) were wheelchair bound, and 2 others in family 2 (patients III:3 and III:6) needed a cane. Seizures occurred in 8 (80%) of the 10 affected members. All 8 individuals had generalized motor seizures. Four of them also experienced complex partial seizures, which tended to occur more frequently than generalized motor seizures and sometimes resulted in secondary generalization of the seizure, while the remaining 4 denied symptoms suggestive of complex partial seizures. Although 1 patient in family 2 (patient III:8) who developed ataxia at the age of 35 years had a history of generalized motor seizures since the age of 15 years, the remaining 7 patients developed seizure disorders within 1 to 4 years after the onset of ataxia (Table). One patient in family 2 (patient III:3) who has been free of seizures for more than 1 year since the onset of ataxia had normal EEG findings, although she has been taking primidone, prescribed by her local physician, for her tremor. In 6 of the 8 patients, seizures were well-con-

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*SCA 10 indicates spinocerebellar ataxia type 10; MRI, magnetic resonance imaging; CT, computed tomographic; EEG, electroencephalographic; EMG, electromyographic; NCV, nerve conduction velocity; PC, complex partial; GM, generalized motor; nl, normal; CSF, cerebrospinal fluid; and ellipses, data not applicable.
†None of the seizures have been monitored.
‡This is the only patient without seizures who underwent EEG.
§This was detected only by examination.
trolled by antiepileptic drugs, such as carbamazepine, phenytoin, and valproic acid. None of the patients who took therapeutic doses of antiepileptic drugs reported noticeable changes in their ataxic symptoms. In 2 patients in family 2 (patients III:0 and III:1), the seizures were not adequately controlled because of poor compliance and alcoholism, respectively. Patient III:0 was recently hospitalized for an episode of status epilepticus. A magnetic resonance imaging scan of the brain in 4 patients in family 2 (patients III:0, III:6, III:8, and III:9) showed a variable degree of pancebellar atrophy without atrophy of the brainstem or cerebral hemispheres (Figure 2). The results of an EEG were normal in 4 patients in family 2 (patients III:3, III:4, III:6, and III:9), but patient III:0 showed bilateral epileptiform discharges. The results of electromyography and nerve conduction studies were normal in 4 patients in family 2 (patients III:1, III:3, III:4, and III:6). Patient III:1 had a long history of alcoholism and proximal muscle weakness, but in addition to the normal electromyographic/nerve conduction velocity study results, he had a normal serum creatine kinase level and normal biceps muscle biopsy findings.

**GENOTYPE ANALYSES**

The Table summarizes the clinical manifestations and genotypes of 22 symptomatic individuals. All affected individuals carried an expansion mutation, with a range of ATTCT repeats from 800 to 4500. The mean±SD of expanded alleles was 2150±1305 (range, 1220–4500) and 2171±973 (range, 800–2820) ATTCT repeats in affected members of families 1 and 2, respectively.

In family 1, the intergenerational change in the size of the expanded ATTCT repeat was a mean±SD gain of 1940±1332 repeats, while the age of onset was a mean±SD of 18.4±4.0 years younger in the offspring than in the transmitting parent (Table, Figure 1A, and Figure 3A and B). Thus, there was a strong inverse correlation between age of onset and the size of the expansion allele ($r^2=0.79$, $P=0.001$). The 4 paternal transmissions in this pedigree resulted in an average net gain of 2425 repeats (range, 1120–3140 repeats), while 1 maternal transmission was associated with no change in the repeat size (from patient III:6 to IV:5 in family 1). An additional 3 maternal transmissions to asymptomatic offspring showed only small repeat size changes (≤40 repeats) (data not shown). Furthermore, the patient (patient IV:3 in family 1) with the earliest onset of disease (at the age of 12 years) in this family acquired the expanded allele of 4100 through a paternal transmission.

In family 2, the mean±SD age of onset of ataxia was 8.7±4.3 years younger in the offspring than in the 2 transmitting fathers (Table, Figure 1B, and Figure 3B). Surprisingly, the intergenerational change in the size of the expanded ATTCT repeat showed a mean±SD loss of 755±703 repeats (Table, Figure 1B, and Figure 3B). All transmissions from these 2 male subjects resulted in contractions of the expanded alleles. Consequently, there was no correlation between age of onset and the size of the expansion allele in family 2.
Initially, it was reported that the disease in family 1 best fit into the category of ADCA type III, because symptoms and signs seemed to be restricted to the cerebellum. Although seizures in 2 patients were noted, neurocysticercosis is endemic in this population and is always a potential cause of seizures. Therefore, whether seizures were a feature of SCA10 was unclear. However, a high prevalence of seizures in the patients of family 2 suggested that the combination of relatively pure cerebellar ataxia and seizures is a unique feature of SCA10. There is reliable evidence that 2 more affected members in family 1 have or had a seizure disorder. In 1 patient in family 1 (patient II:2), a cranial computed tomographic scan showed 1 cyst typical of calcified inactive neurocysticercosis, but this is unlikely to be the cause of her seizures. Seizures have contributed to the morbidity and mortality of the disease. In 2 patients in family 1, who died and where reliable information existed about the cause of death, seizures were a significant contributing factor. One patient in family 2 has been hospitalized for status epilepticus. However, conventional anticonvulsant therapies adequately control the seizure disorders in most patients with SCA10 if compliance can be achieved.

Overall, there is a seizure frequency of 50% (11 of 22 patients) in our patients with SCA10. In comparing the 2 large families with SCA10, the frequencies of seizures were significantly different (3 [25%] of 12 patients in family 1 vs 8 [80%] of 10 patients in family 2; P = .01). There was no correlation between the seizure phenotype and the ATTCT repeat expansion size. Thus, family-specific factors, in addition to the repeat expansion itself, may contribute to the expression of seizure phenotype in individuals with SCA10. Because of the variability of seizure prevalence, the absence of seizures may not exclude the diagnosis of SCA10, especially in relatively small families. Nevertheless, from a practical clinical standpoint, when relatively pure cerebellar signs and symptoms are variably associated with seizures in a patient, DNA testing for SCA10 should be justified for diagnosis.

The mechanism by which the SCA10 mutation leads to the seizure disorder remains unknown. Although imaging studies of the brain of patients with seizures from both families consistently exhibited cerebellar atrophy, they failed to show any evidence of extracerebellar abnormalities that can account for the seizure disorder. Gait ataxia is the first symptom in most patients. It is interesting that seizures have developed in a condition in which all patients universally experience neurodegeneration, apparently confined to the cerebellum. This structure is not typically thought of as involved in the cause of epilepsy, although there have been reports of seizures of cerebellar origin. Alternatively, the seizures may indicate that the neurodegeneration is not restricted to the cerebellum and may be more widespread. Focal EEG abnormalities and the mental changes seen in our patients have also been found in patients with SCA10 in Mexico, 4 and may support the latter possibility.

Four smaller families with SCA10 from Mexico have recently been described; they have a wider spectrum of the clinical phenotype, including a low IQ, sensory polyneuropathy, subtle pyramidal signs, and possible hepatic and hematologic abnormalities, with high interfamilial variability. Families 1 and 2 described herein did not show these findings despite our careful clinical evaluation, except that 2 patients in family 2 (patients III:0 and III:1) had a mild elevation of serum hepatic enzyme levels. Thus, the variability of the SCA10 phenotype is present not only in families 1 and 2 but also among other families with SCA10; this further supports the hypothesis that family-specific factors modify the phenotypic expression of the SCA10 mutation.

Anticipation is a biological phenomenon in which there is an increase in severity or a decrease in the age of onset of a genetic disease with successive generations, and is commonly observed for the trinucleotide repeat disorders. Intergenerational mutant allele instability is a feature common to many trinucleotide repeat disorders and provides the molecular basis for this phenomenon. In family 1, the possible presence of anticipation was reported. Although less striking, family 2 also exhibited anticipation. In family 1, anticipation was accompanied by intergenerational gains of the ATTCT repeat units (Table, Figure 1A, and Figure 3A and B), giving rise to the strong inverse correlation between ATTCT repeat size and the age of onset (R² = 0.79, P < .001). In this family, there may be a difference depending on the sex of the parent transmitting the mutant allele, with further expansions of expanded ATTCT repeat alleles through paternal transmissions, in contrast to no gain or loss of repeat units through maternal transmissions. Studies of additional paternal transmissions in this and other families are needed to confirm this observation. Surprisingly, 2 affected fathers in family 2 transmitted smaller alleles to their offspring. The mechanism of the discrepancy in the directionality of the ATTCT repeat instability remains unclear. The paternal expansion alleles were much larger in family 2 (2780 repeats in patient II:1 and 2820 repeats in patient II:2) than in family 1 (1380 repeats in patient III:1 and 1360 repeats in patient III:3). Although the small sample size does not allow for any conclusions, it raises the possibility that there is an expansion limit in the male germline. Alternatively, family-specific cis- or trans-acting factors may determine the directionality of the ATTCT repeat instability in male germlines. These factors may also affect the postzygotic instability of expanded ATTCT repeats in somatic tissues in parent and offspring. The paradoxical association of intergenerational contractions of the expanded ATTCT repeat alleles with clinically observed anticipation in family 2 is even more puzzling. However, a similar paradox has been observed in myotonic dystrophy type 1, in which the postzygotic somatic expansion of the blood allele in the transmitting father was substantially greater than that of his offspring. This extraordinary somatic expansion of the father’s allele gave rise to the apparent intergenerational contraction of the repeat size when the blood alleles of the father and the offspring were compared. In such cases, the estimated progenitor allele size in the father was actually smaller than that of his offspring, accounting for the clinically observed anticipation. More families and transmissions, with investigations of the germline and somatic instability of their ATTCT repeats, would provide further insight into the genotype-phenotype correlations.
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