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Mapping of a New Autosomal Dominant Spinocerebellar Ataxia to Chromosome 22

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Summary

The autosomal dominant cerebellar ataxias (ADCAs) are a clinically and genetically heterogeneous group of disorders. The clinical symptoms include cerebellar dysfunction and associated signs from dysfunction in other parts of the nervous system. So far, five spinocerebellar ataxia (SCA) genes have been identified: SCA1, SCA2, SCA3, SCA6, and SCA7. Loci for SCA4 and SCA5 have been mapped. However, approximately one-third of SCAs have remained unassigned. We have identified a Mexican American pedigree that segregates a new form of ataxia clinically characterized by gait and limb ataxia, dysarthria, and nystagmus. Two individuals have seizures. After excluding all known genetic loci for linkage, we performed a genomewide search and identified linkage to a 15-cM region on chromosome 22q13. A maximum LOD score of 4.3 (recombination fraction 0) was obtained for D22S928 and D22S1161. This distinct form of ataxia has been designated "SCA10." Anticipation was observed in the available parent-child pairs, suggesting that trinucleotide-repeat expansion may be the mutagenic mechanism.

Introduction

The autosomal dominant cerebellar ataxias (ADCAs) are a clinically heterogeneous group of disorders characterized by ataxia, dysarthria, dysmetria, and intention tremor (Harding 1982, 1993). All ADCAs involve some degree of cerebellar dysfunction and a varying degree of signs from other components of the nervous system. There have been several schemes of classification of the syndromes into different types based on clinical and pathological criteria. One commonly accepted clinical classification (Harding 1993) divides ADCAs into three different groups based on the presence or absence of associated symptoms such as brain-stem signs or retinopathy: presence of pyramidal and extrapyramidal symptoms and ophthalmoplegia (ADCA I), presence of retinopathy (ADCA II), and absence of the associated signs (ADCA III).

Genetic-linkage studies and molecular analysis of the gene mutations have revealed that ADCAs are genetically heterogeneous even within the various subtypes. ADCA I is caused by mutations in three different genes: those for spinocerebellar ataxia (SCA) types 1–3 (SCA1–SCA3) (Orr et al. 1993; Kawaguchi et al. 1994; Imbert et al.1996; Pulst et al. 1996; Sanpei et al. 1996). ADCA II is caused by mutations in SCA7 (David et al. 1997; Del-Favero et al.1998; Koob et al. 1998). All mutations involve a trinucleotide-repeat expansion in the coding region of the gene. ADCA III is a predominant cerebellar phenotype and has been described in one large pedigree with linkage to chromosome 11 and has been designated "SCA5" (Ranum et al. 1994). In addition, CAG expansions in the CACNl gene on chromosome 19 cause SCA6 (Zhuchenko et al. 1997) and, especially in the lower pathological repeat range, cause a predominantly cerebellar phenotype.

Elsewhere we have identified a four-generation Mexican pedigree that segregates a distinct form of SCA (Grewal et al. 1998). The clinical phenotypes are characterized by predominantly cerebellar symptoms and signs and thus fall within the ADCA III clinical classification. Two affected individuals also had seizures, but it could not be determined whether these were caused by focal CNS lesions or were part of the degenerative phenotype. All known SCA loci, as well as mutations in the DRPLA gene, were excluded by direct mutation or genetic-linkage analysis. We now report the results of a genomewide search for linkage and assignment of SCA10 to human chromosome 22q.

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This article is dedicated to Sam Winograd, a friend and a supporter of science for the improvement of mankind.

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Subjects and Methods

The subjects were recruited from a four-generation Mexican family in which a distinct form of autosomal dominant SCA was ascertained, clinically characterized by gait and limb ataxia, dysarthria, and nystagmus. After informed consent (approved by the institutional review boards of the University of Southern California and Cedars-Sinai Medical Center) was obtained, blood samples were drawn from the 20 available family members—10 affected individuals and 10 unaffected individuals. Genomic DNA samples were extracted according to standard protocols (Puregene; Gentra Systems).

We performed a genomewide search for the genetic locus underlying this new form of SCA. A total of 386 fluorescent-tagged microsatallite markers in the Human Screening Set version 8/8aRG were purchased from Research Genetics. These markers cover the entire genome, with an average 10-cM interval.

After mapping of the SCA10 locus, we searched the Sanger Centre sequence database for potential polymorphic genetic markers and designed primers by using the Primer 3 program of the Whitehead Institute for Biomedical Research/MIT Center for Genome Research. One simple-tandem-repeat marker in plasmid-artificial-chromosome (PAC) clone DJ522J7 was informative in the pedigree and was amplified by the following primers: CADJ522 A (5′-aacacggccaaatctcaaac-3′) and CADJ522B (5′-gagggtagaatcaactccatgc-3′).

PCR was performed in a 20-μl reaction containing 100 ng of genomic DNA template, 100 pmol of each primer, 2.5 mM each dNTP, 2.5 mM $MgCl_2$, and 1 unit of Gold *Taq* polymerase (Perkin-Elmer) in the 10 × buffer provided by the vendor. The reaction conditions were as follows: initial denaturation at 94°C for 2 min; 30 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 60 s; and a final extension at 72°C for 7 min (all steps were completed within a 96-well PTC 100 thermal cycler [M.J. Research]). The PCR products were then analyzed in a 6% denaturing polyacrylamide gel, by an Applied Biosystems (ABI) 373 automated DNA sequencer. The markers in the same panel designed by the vendor were pooled and analyzed in one lane. Allele numbers were determined according to the internal standard (Tamra 350; Perkin Elmer/ABI). Both Genescan and Genotyper software packages (Perkin-Elmer/ABI) were utilized for the computer analysis to transfer data from image to digital numbers.

Two-point linkage analysis was performed by use of the linkage 5.1 package (Lathrop and Lalouel 1984), under the assumption of autosomal dominant inheritance and a disease frequency of 1/100,000. The allele frequencies were assumed to be equal for each marker. Penetrance was assumed to be .95. In separate calculations, penetrance was set at .99 in generation II, .90 in generation III, and .80 in generation 4, without significant changes in LOD scores. Similarly, changes in allele frequencies did not have a significant effect on LOD scores.

Results

A summary of the clinical phenotype in this family is shown in table 1 and is notable for the absence of symptoms or signs in other CNS structures, except for the presence of seizures in two individuals. To map this new SCA, we undertook a genomewide search for linkage. After testing 47 markers, we detected a positive LOD score of 1.82, at recombination fraction [θ] .11, with marker D22S683.

We explored this result further by using markers flanking D22S683, to test linkage. Markers proximal to D22S683 recognized additional recombination events (data not shown), whereas several markers distal to D22S683 yielded higher LOD scores (table 2). Markers D22S928 and D22S1161 were informative for each meiosis and yielded the highest LOD scores: 4.30. Changing the penetrance assumptions or allele frequencies had only minimal effects on the observed LOD scores (data not shown). Even at a penetrance of .50, all maximum LOD score (Z_{max}) values were still >3.

No telomeric recombination events were identified for chromosome 22 markers available from the public genetic map. We therefore searched for extended CA repeats in the genomic sequence database at the Sanger Centre. Several such repeats were identified in PAC clones mapping telomeric on chromosome 22. We tested these in the pedigree described above and in 10 unrelated individuals. One marker, CADJ522, which was identified from clone DJ522J7, was informative in the pedigree and detected one recombination event in an affected individual. According to the physical map from the Sanger

Table 1

Clinical Characteristics of Patients with SCA10

Symptom(s) or sign(s)	Incidence (%)
Truncal and gait ataxia	100
Dysarthria	100
Nystagmus	100
Seizures	20
Pyramidal or basal-ganglion signs	0
Dementia	0
Ophthalmoplegia or visual loss	0
Sensory loss	0

Centre, there are three overlapping and completely sequenced PAC clones between clone DJ522J7 and clone DJ170A21, which contains the sequence for genetic marker D22S526, the last distal marker available from the public genetic map. The physical distance between D22S526 and CADJ522 is ~520 kb. Thus, the SCA10 candidate region encompasses ~15 cM flanked proximally by D22S1140 and distally by CADJ522 (fig. 1).

Haplotypes for the markers are shown in figure 2. For D22S683, the first marker showing linkage in the initial genome scan, two recombination events were detected in individual IV:1 (affected) and in individual III:7 (unaffected). For D22S1140, a recombination event was identified in individual IV:1; and, for CADJ522, a recombination event was identified in individual IV:2. Both of these individuals are clearly affected.

Anticipation was observed in the age at onset in this pedigree (fig. 2). In generation III, the average age at onset was 34.2 years, and in generation IV it was 14.5 years; the difference was significantly different ($P <$ .0002). In this pedigree, the Penrose coefficient calculated for individuals in generations III and IV was .84, which is similar to the coefficient calculated for pedigrees with myotonic dystrophy (Penrose 1948).

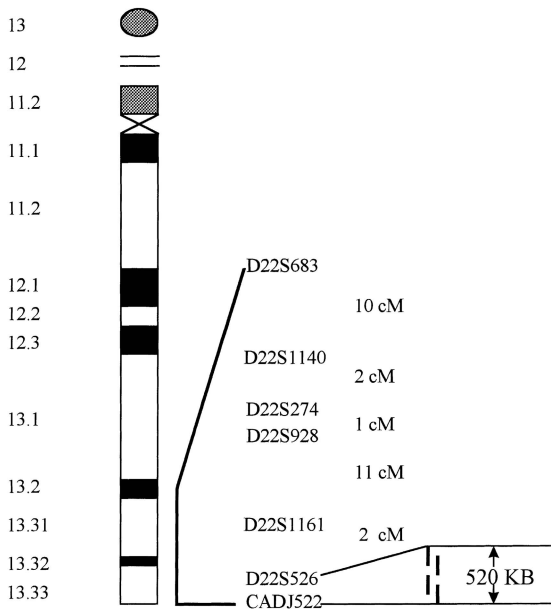

Figure 1 Ideogram of chromosome 22, showing markers linked to SCA10, as well as their relative genetic or physical distances (from Genome Database). Marker CADJ522 was identified by searching the Sanger Centre database. The four PAC clones between marker D22S526 and CADJ522 have all been completely sequenced and span a distance of 520 kb.

Discussion

We have identified a new SCA locus on human chromosome 22; in addition to five identified autosomal dominant ataxia genes and two SCA loci, we have here presented evidence for an additional ataxia locus, which we have designated "SCA10." On the basis of the phenotype in this SCA10 family, as summarized in table 1, SCA10 is associated with an ADCA III phenotype. ADCA III refers to a phenotype that is purely or predominantly cerebellar in nature. However, additional families linking to the SCA locus will need to be assessed to determine the phenotypic spectrum of SCA10, especially with regard to the presence of seizures. For example, SCA6 phenotypes vary from ADCA type III for moderate expansions to an ADCA type I phenotype with longer CAG expansions.

It is interesting to note that no new SCA loci have been mapped by genetic-linkage analysis during the past 3 years. The last chromosomal assignment of an ADCA was in 1995, when SCA7 was mapped to human chromosome 3p (Benomar et al. 1995; Gouw et al. 1995; Holmberg et al. 1995; David et al. 1996; Krols et al. 1997). This is despite the fact that one-third of ADCA pedigrees worldwide have not been assigned to known genes or loci (Ranum et al. 1995; Geschwind et al. 1997;

Table 2

Two-Point LOD Scores for SCA10 versus Markers in chromosome 22q13

	LOD Score at θ =								
Locus	0	.01	.05	.10	.20	.30	.40	Z_{max}	Maximum θ
D22S683	−3.6	1.02	1.68	1.82	1.62	1.17	.56	1.82	.11
D22S1140	−3.96	1.87	2.32	2.29	1.90	1.31	.60	2.34	.07
D22S274	3.98	3.92	3.65	3.29	2.54	1.70	.79	3.98	0
D22S928	4.30	4.22	3.94	3.56	3.16	1.86	.87	4.30	0
D22S1161	4.30	4.22	3.94	3.56	3.16	1.86	.87	4.30	0
D22S526	2.25	2.21	2.04	1.82	1.37	.87	.34	2.25	0
CADJ522	−4.65	.19	.77	.90	.86	.65	.36	.92	.13

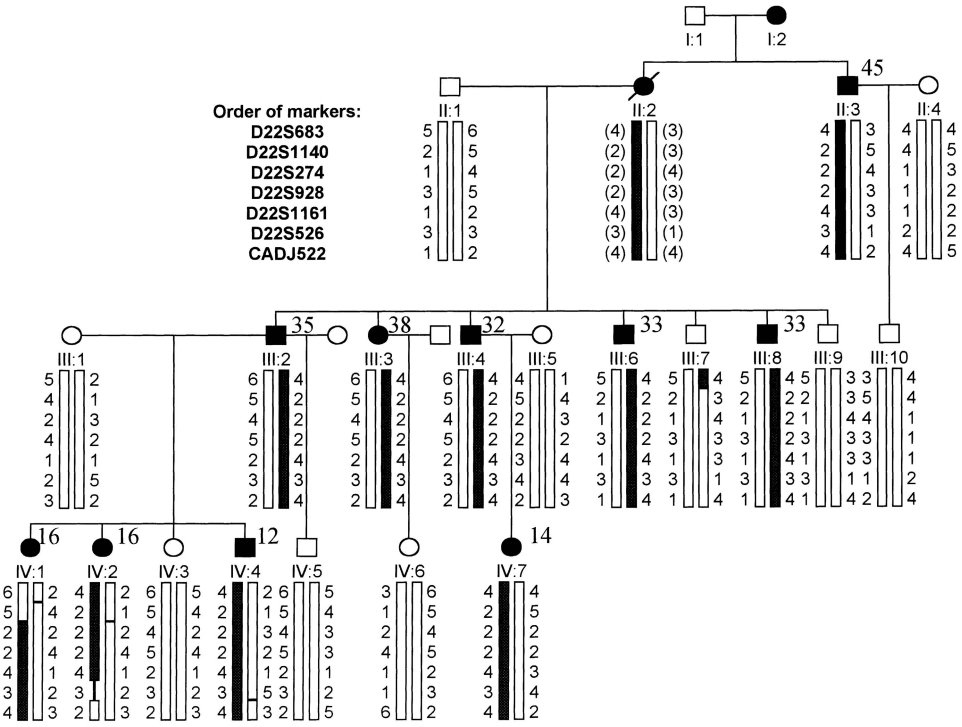

Figure 2 Haplotypes for seven chromosome 22 markers in the Mexican-American SCA10 pedigree. Note the recombination events in III:7, IV:1, and IV:2. Affected individuals are represented by blackened symbols, unaffected individuals are represented by unblackened symbols, and the diagonal slash through the symbol for II:2 denotes that this individual is deceased. Ages at onset are given at the upper right of the symbols.

Riess et al. 1997; and Schols et al. 1997). It is possible that, in the existing pedigrees, significant anticipation or disease severity has resulted in a pedigree too small for genetic-linkage analysis. Alternatively, significant segregation distortion could influence pedigree size. The identification of the SCA10 locus now makes it possible to examine linkage in these smaller pedigrees. We have since tested two unassigned smaller pedigrees from the University of California, Los Angeles, clinic. Both were not linked to the SCA10 locus (data not shown).

Except for questionable anticipation in SCA4 (Flanigan et al. 1996), all dominant SCAs have shown anticipation. The analysis of a pedigree for the presence of anticipation may be difficult, because, in the youngest generation, asymptomatic gene carriers who have not yet developed the disease may bias ascertainment. After close flanking markers have been identified, this bias can be excluded. Indeed, in the Mexican SCA10 pedigree, linkage analysis confirmed that all individuals with the disease haplotype had already developed symptoms. Anticipation in this pedigree appears to be marked and occurred at age 18–23 years (fig. 2). Unlike SCA5, in which the most dramatic examples of anticipation were seen with maternal transmissions, anticipation in SCA10 appears to occur in paternal transmissions, although, clearly, a larger number of transmissions need to be studied. These observations suggest that SCA10, like the other SCAs, may be caused by an unstable trinucleotide repeat. This could be tested by the repeat expansion–detection method (Schalling et al. 1993) or by use of a monoclonal antibody that detects expanded glutamine repeats (Trottier et al. 1995). Both methods, however, require significant polyglutamine expansions.

The 15-cM region on chromosome 22 covers an estimated 9-million-bp physical distance, according to the physical map from the Sanger Centre. Despite this rather large physical distance, positional cloning of SCA10 can be achieved. First, this part of the genome has been intensively investigated by the Human Genome Project. So far, ~90 clones (bacterial artificial chromosome, PAC, and cosmid) have been identified that cover this entire region, leaving few gaps. Of 90 clones, half have been completely sequenced, and many are partially sequenced. Second, both the observation of anticipation in the age at onset in the pedigree and the fact that all the SCA mutations thus far identified involve a trinu-

cleotide-repeat expansion suggest that SCA10 may contain a trinucleotide repeat within its sequence. Identification of another SCA gene will greatly facilitate diagnosis of SCA patients and also will have an impact on our understanding of the molecular mechanisms underlying ADCAs.

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Electronic-Database Information

URLs for data in this article are as follows:

Genome Database, http://gdbwww.gdb.org
Sanger Centre, http://www.sanger.ac.uk
Whitehead Institute for Biomedical Research/MIT Center for Genome Research, http://www.genome.wi.mit.edu (for Primer 3 program)

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