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Confirmation of the *SCA-2* Locus as an Alternative Locus for Dominantly Inherited Spinocerebellar Ataxias and Refinement of the Candidate Region

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Summary

The autosomal dominant spinocerebellar ataxias (SCAs) are a clinically heterogeneous group of neurodegenerative diseases. To date, two SCA loci have been identified—one locus (*SCA-1*) on the short arm of chromosome 6 and the second locus (*SCA-2*) on the long arm of chromosome 12. We have studied two large kindreds from different ethnic backgrounds, segregating an autosomal dominant form of SCA. A total of 207 living individuals, including 50 affected, were examined, and blood was collected. We performed linkage analysis using anonymous DNA markers which flank the two previously described loci. Our results demonstrate that the two kindreds, one Austrian-Canadian and one French-Canadian, are linked to *SCA-2* (chromosome 12q). Multipoint linkage analysis places the *SCA-2* locus within a region of approximately 16 cM between the microsatellites D12S58 and D12S84/D12S105 (odds ratio 2,371:1 in favor of this position). We show that the *SCA-2* locus is not a private gene and represents an alternative SCA locus.

Introduction

The spinocerebellar ataxias (SCAs) represent a wide spectrum of neurodegenerative diseases. Although epidemiologic studies of these disorders are few and geographically limited (Barbeau 1978; Skre 1978; Werdelin and Keiding 1990), a European survey suggests that the prevalence of familial cases, usually autosomal dominant with age-dependent penetrance, is 1.1 per 100,000 population (Gudmundsson 1969). The variety of symptoms observed and the intra- and interfamilial variability in age at onset and both neuropathological and biochemical findings underscore the clinical heterogeneity (Greenfield 1954; Harding 1984). Despite extensive clinical, epidemiological, pathological, and neurochemical studies, the pathophysiological mechanisms underlying the various forms of SCA remain unknown (Duvoisin et al. 1983; Kish et al. 1987; Blass et al. 1988; Manyam et al. 1990; Pedraza and Botez 1992). It is now generally accepted that the diagnostic controversies involving this group of disorders will only be resolved when the locus or loci are identified, genes are cloned, and mutations are determined (Rosenberg 1990).

In 1974, Yakura et al. (1974) demonstrated linkage between one SCA pedigree of Japanese origin and the HLA locus on the short arm of chromosome 6 (ch 6p), the *SCA-1* locus. The gene defect has now been more precisely mapped on ch 6p in several families (Jackson et al. 1977, 1978; Moller et al. 1978; Koeppen et al. 1980; Nino et al. 1980; Haines et al. 1984; Kumar et al. 1986; Rich et al. 1987; Frontali et al. 1991), and a can-

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didate mutation subsequently has been identified (Orr et al. 1993). However, linkage was excluded in an approximately equal number of pedigrees, thus confirming the existence of locus heterogeneity (Wastiaux et al. 1978; Pedersen et al. 1980; Auburger et al. 1990; Carson et al. 1992; Ranum et al. 1992; Lazzarini et al. 1992; Khati et al. 1993). Recently, a second locus, *SCA-2*, was assigned to a 36-cM interval of the long arm of chromosome 12 (ch 12q) in a large cluster of Cuban families showing a clear founder effect (Gispert et al. 1993). Because the Cuban kindred is part of a very inbred population (Orozco-Diaz et al. 1990), this newly described *SCA-2* locus could represent a private gene, specific for that particular kindred. Therefore, the *SCA-2* locus needs to be confirmed as a possible alternative locus for dominant non–*SCA-1* families. Recently, a clinically similar disorder, Machado-Joseph disease, has been assigned to the long arm of chromosome 14 (ch 14q) in Japanese families with Machado-Joseph disease (Takiyama et al. 1993).

It is important to test and report all available informative SCA families, in order to determine the relative frequencies and ethnic origins of families which map to ch 6p, ch 12q, and elsewhere, as well as to identify clinical differences among these families. This approach will facilitate efforts to map all SCA loci, thereby eventually improving the classification and understanding of this group of conditions. With these aims in mind, we tested two large families for linkage to ch 6p and 12q markers.

Subjects and Methods

Patient Material

A total of 207 living individuals from two SCA kindreds, including 50 affected individuals, were ascertained. Each kindred was examined during field trips to the regions where the majority of family members live. All individuals were seen by the same group of investigators (I.L.-C., F.C., F.A., and E.A.), which facilitated uniform clinical reporting and improved the assessment of clinical variability within and between families. In addition, medical records from all affected and possibly affected individuals were reviewed. Four patients with various ages at onset and clinical progression were admitted to the Montreal Neurological Hospital for detailed neurological and neuroophthalmological investigation. The two kindreds showed a clear autosomal dominant pattern of inheritance and no evidence for incomplete penetrance. Table 1 summarizes the main clinical features.

The Saskatchewan-Austrian kindred (SAK).—This kindred (fig. 1*A*) is of Austrian background. The first ancestral couple came to Canada in 1910 and established themselves as farmers on the prairies. Relatives remained in Austria, and we have identified several branches of this family who suffer from the disease and who are still living in a small village between Vienna and the Austro-Hungarian border (Gerstenbrand and Weingarten 1962). Seventy-five living individuals in the Saskatchewan branch, with 20 affected, and 68 living individuals in the Austrian branches, with 12 affected, were examined. The mean age at onset of symptoms in living and deceased individuals combined was 34.3 years, with a range of 15–60 years. The first symptoms were poor balance with unsteadiness of gait. In several patients, muscle cramps were an initial finding. Abnormal eye movements were present in the majority of the affected individuals; these were not detected by routine neurological examination in only six ataxic individuals. Four affected individuals from the Saskatchewan branch were assessed by neuroophthalmological examination, which showed full range of pursuit eye movements, except for slightly limited upward gaze; however, the saccades were extremely slow in all four patients, with a range of 100–300 degrees/s. Almost half of the patients had fasciculations of the trunk and/or limbs. This family showed remarkable clinical heterogeneity among affected individuals. Some patients, mainly those with later age at onset, had slow progression of the disease with predominantly cerebellar signs, whereas those with earlier age at onset had a much faster evolution and more widespread involvement of the central nervous system.

The Gaspé kindred (GK).—This kindred (fig. 1*B*), one of the largest SCA pedigrees ever described (Barbeau 1978; Wastiaux et al. 1978), is of French ancestry. The first ancestors came to Quebec in 1836 and established themselves in the Gaspé peninsula. They were originally from the northern coast of France, probably Brittany. A total of 64 living individuals, including 18 affected, were examined. The mean age at onset of symptoms for living and deceased individuals combined was 31 years, with a range of 14–50 years. The first symptom was usually unsteadiness of gait. Muscle cramps and fasciculations were present in the majority of affected individuals. On neurological examination, slow saccades were present in all but one affected individual. Progression of symptoms was variable: some patients were only mildly disabled in their late 60s, while others were already severely handicapped in their early 40s.

Table 1

Clinical Features in Two SCA Families

Kindred (*n*)	Mean Age at Onset[a] (years)	No. (%) of Individuals with									
		Ataxia	Abnormal Eye Movement	Perioral Fasciculation	Head Tremor	Dysarthria	Dysphagia	Fasciculations of Trunk/Limbs	Decreased Reflexes	Increased Reflexes	Impairment of Vibration
SAK (32)	36.8	32 (100)	26 (81)	22 (69)	13 (41)	24 (75)	12 (38)	13 (41)	15 (47)	9 (28)	11 (58)
GK (18)	28.8	18 (100)	17 (94)	15 (83)	9 (50)	14 (77)	9 (50)	11 (61)	9 (50)	5 (27)	10 (55)

NOTE.—The figures represent the number of individuals who had the stated clinical features at the time of neurological examination. Percentages shown in brackets represent the number of individuals with a specific clinical finding divided by the total number of affected individuals examined in each family.

[a] Refers only to patients examined.

Methods

Thirty to forty milliliters of venous blood were obtained from each individual in glass tubes containing acid citrate dextrose. Ten milliliters were processed to establish Epstein-Barr virus–transformed lymphoblastoid cell lines, in order to provide an inexhaustible source of DNA and a living cell line for future use (Anderson and Gusella 1984). The remaining blood was used for genomic DNA preparation by direct extraction from lymphocytes using standard manual techniques (Sambrook et al. 1989).

Typing with microsatellite markers.—A total of 123 individuals, including 50 affected, were typed with polymorphic dinucleotide or trinucleotide repeat sequences which flank the two previously described SCA loci, *SCA-1* and *SCA-2*. We used a polymorphic dinucleotide repeat at the D6S89 locus, which is very close to the *SCA-1* locus on ch 6p (Litt and Luty 1990; Keats et al. 1991; Ranum et al. 1991; Wilkie et al. 1991; Zoghbi et al. 1991). The *SCA-2* locus was assigned to ch 12q23-24.1 (Gispert et al. 1993) within a large region between two microsatellites at the D12S58 and PLA 2 loci (Polymeropoulos et al. 1990; Nancarrow et al. 1992). In order to completely cover the candidate region, we tested these two markers and three additional ones—D12S84 and D12S105, which have been mapped within the interval containing the SCA 2 locus, and D12S76, which is telomeric to the PLA 2 locus (Généthon 1992; Weissenbach et al. 1992). Using the published primer sequences, PCR was carried out in a total volume of 12.5 μl containing 40 ng genomic DNA; 125 ng of each primer; 200 μM each of dGTP, dCTP, and dTTP; 25 μM dATP; 1.5 μCi [^{35}S] dATP; 0.5 units of *Taq* DNA polymerase (Bio/Can Scientific); and 2.0 μl of 10 × buffer (Bio/Can Scientific) with $MgCl_2$ included in the final concentration of 1.5 mM. Samples were overlaid with mineral oil and processed throughout 30–35 cycles of denaturation, annealing, and elongation at different temperatures optimized for each marker. PCR products were analyzed on 6% denaturing polyacrylamide gel (38:2 acrylamide:bisacrylamide). Samples were run for an average period of 2 h in a vertical electrophoresis gel apparatus (Life Technologies). Gels were dried and exposed to X-ray films for 48–72 h at room temperature.

Data analysis.—Linkage between the SCA locus in the pedigrees and each of the markers on ch 6p and ch 12q was analyzed by the maximum-likelihood method using the computer program LIPED (Ott 1974). To simplify analysis, the Austrian-2 branch of the SAK (see fig. 1*A*) was analyzed as a separate family. We used an age-at-onset correction function that assumed (i) a normal distribution with a mean age at onset of the disease derived from each pedigree and (ii) a step function based on five age intervals (Hodge et al. 1979). An autosomal dominant transmission and a mutant allele frequency of 1:100,000 were assumed. The gene frequency for each marker allele was calculated from the unrelated individuals married into the pedigrees. In order to further narrow the candidate region on ch 12q, we carried out a multilocus linkage analysis on each pedigree using the LINKMAP program (version 5.1) from the LINKAGE package (Lathrop et al. 1984). Age-dependent penetrance of the disease, using five different liability classes, was also considered. Mode of inheritance, mutant gene frequency, and marker allele frequencies were the same as described above. In order to simplify the calculations, the markers D12S84 and D12S105 were analyzed as a haplotype, since they are tightly linked (Généthon 1992; Weissenbach et al. 1992). By convention, the test for linkage is declared significant when the maximum lod score (Z_{max}) exceeds the bound $Z_0 = 3.00$. Conversely, values of recombina-

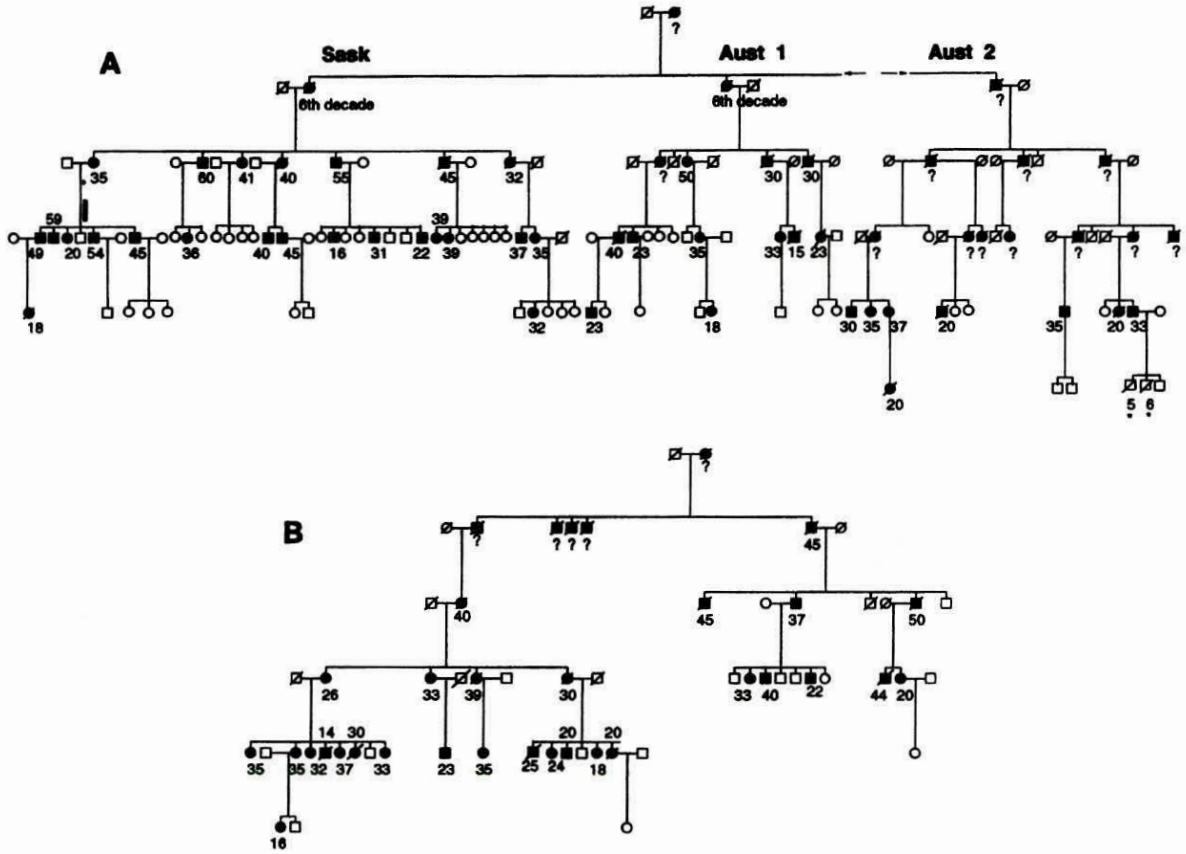

Figure 1 *A*, Reduced pedigrees of SAK. Sask = Saskatchewan branch; Aust 1 = Austrian branch 1; and Aust 2 = Austrian branch 2. The relationship of Aust 2 with the rest of the family dates back to at least three generations before the founder couple represented. *B*, Pedigree of GK. Blackened symbols represent affected individuals. Diagonal lines indicate deceased individuals. Numbers placed above or below blackened symbols indicate the approximate age at onset of symptoms. When age at onset was not known, a question mark (?) was placed there instead. An asterisk (*) indicates two individuals in the Aust 2 branch who died around 12 years of age, after suffering for 6 years of a progressive disease and for whom no clinical information is available. All 123 living individuals in the two kindreds shown in the pedigrees were included in the linkage analyses.

tion fraction (θ) at which $Z_\theta < -2.00$ are said to be definitively excluded (Ott 1991).

Results

Lod Score on Ch 6p

The two-point lod scores obtained between the disease locus and the D6S89 marker are summarized in table 2. The lod scores were significantly negative for both kindreds, with exclusion ($Z < -2.00$) up to θ = .20 and .04 in SAK and GK, respectively.

Lod Scores on Ch 12q

The two-point lod scores between each of the ch 12q markers and the disease locus in each kindred are summarized in table 2. In both kindreds, SAK and GK, positive lod scores were obtained: $Z_{max} = 6.320$ ($\theta = .059$) with the D12S105 marker for SAK, and $Z_{max} = 2.111$ ($\theta = .215$) with the PLA 2 marker for GK.

Four-point lod scores obtained against the markers on ch 12q for SAK and GK revealed the following: for SAK alone, $Z_{max} = 11.770$ was obtained for the location of the *SCA-2* locus in the interval between the D12S58 and D12S84/D12S105 loci. A second peak, $Z_{max'} = 8.579$, was obtained when the *SCA-2* locus was placed between D12S84/D12S105 and PLA 2. In GK, $Z_{max} = 5.856$ was obtained when the *SCA-2* locus was placed between the D12S58 and D12S84/D12S105 loci, and a second peak, $Z_{max'} = 5.315$, was obtained in

Table 2

Two-point Lod Scores Obtained with Ch 6p and 12q Markers

Kindred and Repeat Marker[a]	Z at θ = .01	.05	.10	.15	.20	.25	Z_{max}	θ
SAK:								
D6S89	−9.830	−5.627	−3.819	−2.769	−2.215	−1.482	...	...
D12S58	.305	1.434	1.676	1.686	1.555	1.369	1.701	.160
D12S84	1.956	4.341	4.088	3.271	2.297	1.987	4.373	.059
D12S105	2.103	6.231	5.776	4.525	3.103	2.001	6.320	.059
PLA 2	.281	.932	1.268	1.754	2.399	1.705	2.405	.230
D12S76	.548	.967	1.875	1.989	2.112	2.249	2.412	.270
GK:								
D6S89	−3.179	−1.748	−.998	−.620	−.425	−.242	...	...
D12S58	−.497	.679	.996	1.055	1.005	.893	1.055	.150
D12S84	1.132	1.771	1.698	1.241	.967	.651	1.776	.053
D12S105	.875	1.297	1.189	1.035	.809	.641	1.302	.053
PLA 2	.794	1.601	1.894	2.043	2.104	1.852	2.111	.215
D12S76	.457	.783	.978	1.197	1.166	1.404	1.404	.250

[a] Dinucleotide or trinucleotide repeat markers.

the alternative location, between D12S84/D12S105 and the PLA 2 locus. When the two kindreds, SAK and GK, were combined (fig. 2), Z_{max} = 15.742 was achieved in the centromeric position, between the D12S58 and D12S84/D12S105 loci, whereas a second peak, Z_{max} = 12.367, was obtained in the telomeric position, between the D12S84/D12S105 and the PLA 2 locus. No significant sex differences in recombination fractions were found in either two-point or four-point analyses.

Discussion

Exclusion of linkage to ch 6p and positive linkage of both SAK and GK to ch 12q clearly establish that both families map to the *SCA-2* locus. We have shown that families of different ethnic backgrounds, SAK and GK, of Austrian and French ancestry respectively, map to the *SCA-2* locus on ch 12q, which was originally described only in a large, homogeneous Cuban kindred of Spanish ancestry. Therefore the ch 12q SCA locus, *SCA-2*, is not a private gene and may represent a major SCA locus. Furthermore, since the ethnic origins of these families are different, they may represent different mutations at the *SCA-2* locus.

The highest maximum lod score for both GK and SAK was obtained in the 16-cM interval flanked by the microsatellites at the D12S58 and D12S84/D12S105 loci (combined Z = 15.742). The next highest peak, in the interval flanked by the D12S84/D12S105 and PLA 2 loci, yields combined Z = 12.367. Therefore, the position of *SCA-2* in the interval D12S58–D12S84/D12S105 is favored by odds of 2,371:1. These results lead to a more precise localization of the *SCA-2* locus within the candidate region previously determined by Gispert et al. (1993).

Our findings confirm locus heterogeneity among the dominant forms of SCA (Wastiaux et al. 1978; Pedersen et al. 1980; Auburger et al. 1990; Carson et al. 1992; Lazzarini et al. 1992; Ranum et al. 1992; Khati et al. 1993). To date, no clinical features have been identified which may be considered characteristic for one or the other SCA type. However, the similar clinical findings in SAK and GK suggest a possible uniform clinical picture in the *SCA-2* families. For example, although the range in age at onset of symptoms is quite broad in both families, the clinical phenotypes vary according to age at onset of symptoms: individuals with early onset have faster progression of symptoms and more widespread disease of the CNS, whereas individuals with late onset seem to develop mainly a pure cerebellar syndrome. One characteristic clinical finding, which was present in the majority of affected individuals, is the slowness of saccades in the presence of almost normal pursuit eye movements. We also found that the presence of lower motor neuron involvement, which is not a prominent feature of SCA in general (Harding 1984), was very common in our *SCA-2* kindreds. Several clinical characteristics of our two *SCA-2* kindreds, SAK and GK,

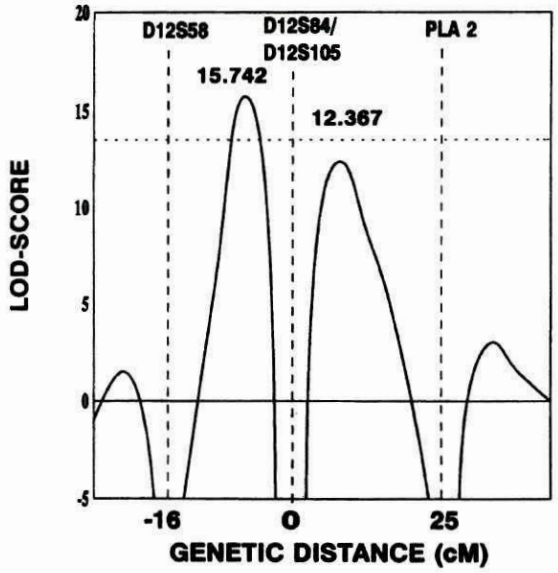

Figure 2 Four-point lod scores obtained with markers on ch 12q in SAK and GK combined. The markers on the D12S84 and D12S105 loci were analyzed as a haplotype, since these two markers are tightly linked (see text). Genetic distances between markers available on the CEPH families are the following: D12S58–PLA2 = 36 cM (Gispert et al. 1993), D12S84–D12S105 = 0 cM (Weissenbach et al. 1992), and D12S84/D12S105–D12S76 = 17.5 cM (Weissenbach et al. 1992). The following genetic distances were generated from our two *SCA-2* pedigrees: D12S58–PLA2 = 43.4 cM; D12S84–D12S105 = 0 cM; D12S84/D12S105–D12S76 = 30.2 cM; D12S58–D12S84/D12S105 = 15.6 cM; and D12S84/D12S105–PLA2 = 24.9 cM. The most probable order of the markers on ch 12q, as indicated by the data obtained on the CEPH families and the results in our two *SCA-2* pedigrees, is the following: cen–D12S58–D12S84/D12S105–PLA 2–D12S76. Recombination fractions were converted into cM by using the Kosambi map function and are approximated. Zero was arbitrarily placed at the D12S84/D12S105 loci. The horizontal dotted line indicates a lod score value of $Z_{max} - 1.00$.

were strikingly similar to those of the Cuban kindred, such as wide range in age at onset, presence of clinical variability within families, extremely slow saccades, and clinical signs of lower motor neuron disease (Orozco-Diaz et al. 1990). To our knowledge, there is another cluster of SCA families in India, reported by Wadia and Swami (1971), with very similar clinical findings, particularly slow saccades. To date, no linkage data are available in these kindreds. Therefore, our two *SCA-2* pedigrees, with 50 affected individuals, offer a unique opportunity for a detailed study of clinical variability between SCA pedigrees and within *SCA-2* families.

Our data, which significantly refine the placement of the *SCA-2* locus on ch 12q23-24.1.1 and clearly identify flanking markers, represent an important first step in the positional cloning of the gene. Furthermore, the large families reported here and the numerous recombination events should allow even better refinement of the *SCA-2* candidate region as more markers become available.

The more precise localization and the identification of multiple informative flanking markers will facilitate genetic counseling based on linkage data. This will benefit the over 200 at-risk individuals in these two large *SCA-2* kindreds. However, use of these markers for diagnosis in other SCA pedigrees will require careful analysis to confirm *SCA-2* linkage and exclude other SCA loci, in order to avoid errors caused by nonallelic genetic heterogeneity. The highly informative flanking markers reported here should facilitate positive assignment of SCA pedigrees to ch 12q.

Note added in proof.—Recently, another SCA kindred has been assigned to ch 12q (Pulst et al. 1993).

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