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Tight Linkage of the Gene for Spinocerebellar Ataxia to D6S89 on the Short Arm of Chromosome 6 in a Kindred for Which Close Linkage to Both HLA and F13A1 Is Excluded

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Summary

A locus for an autosomal dominant form of spinocerebellar ataxia (SCA1) has been assigned to the short arm of chromosome 6 on the basis of linkage to the major histocompatibility system (HLA). In this study of a five-generation American black family, close linkage between the disease locus and both HLA and the coagulation factor XIIIA (F13A1) locus was excluded, and lod scores for all locations of the disease locus between HLA and F13A1 were less than −1.4. These results suggest that the locus causing spinocerebellar ataxia in this family is not in this region. However, the disease locus was found to be closely linked to a microsatellite polymorphism, D6S89, which is between HLA and F13A1. The maximum lod score for SCA1 and D6S89 is 4.90 at a recombination fraction of 0, both in males and in females. These data show that exclusion of close linkage to the HLA complex and F13A1 in a kindred with spinocerebellar ataxia does not rule out the possibility that the disease locus in that family is on 6p. Accordingly, all families segregating a dominantly inherited ataxia should be evaluated for linkage to D6S89, to determine whether the locus causing the disease is SCA1.

Introduction

The spinocerebellar ataxias (SCAs) are characterized by progressive neurodegeneration in the cerebellum, leading to a typical pattern of gait ataxias and dysarthria in affected individuals. Onset of symptoms is usually in the third to fifth decade, with death occurring 10–15 years after onset. The clinical variability in the associated signs such as external ophthalmoplegia, dementia, hyperreflexia, impaired vibration sense, tremor, myoclonus, distal weakness, spasticity, rate of progression, and age at onset have made classification difficult (Harding 1982; Currier 1984). Precise localization and identification of the gene(s) causing the disease will allow accurate classification of the SCAs.

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Linkage of the gene for one type of the dominantly inherited ataxias to the major histocompatibility complex (HLA) was suggested by Yakura et al. (1974) and confirmed by Jackson et al. (1977). The locus for the HLA-linked form of SCA has been designated SCA1. Studies of additional SCA families showed linkage to HLA in some but not others, although Morton et al. (1980) were not able to detect significant heterogeneity among nine pedigrees. Attempts to localize the gene relative to HLA suggested both a proximal (Zoghbi et al. 1989) and a distal (Rich et al. 1987) location, and a study of several large Cuban pedigrees to try to confirm one or the other of these locations excluded the disease locus from at least 40 cM on either side of HLA (Auburger et al. 1990). Very recently, studies using a dinucleotide repeat polymorphism at the locus D6S89, which is telomeric to HLA (Litt and Luty 1990), demonstrated close linkage of D6S89 to SCA1 in several families for which linkage to HLA was confirmed (Zoghbi et al. 1989, 1991; Ranum et al. 1991). These studies support the assign-

ment of SCA1 to a region telomeric to HLA, on the short arm of chromosome 6.

In the present paper we perform pairwise and multipoint linkage analyses using D6S89 and other genetic markers on the short arm of chromosome 6, in order to map the gene causing dominantly inherited SCA in a five-generation kindred for which close linkage to HLA is excluded.

Material and Methods

The family being studied is of American black ancestry and includes five generations of individuals with SCA. This family is not known to be related to the black family studied by Zoghbi et al. (1989, 1991). Members of this kindred have been examined by one of us (M.A.W.) over a 10-year period, providing detailed neurological documentation of the clinical progression of the disease.

Blood samples for HLA typing, DNA extraction, and separation of hemolysates and plasma were drawn from 21 family members, including eight affected individuals. Typing for antigens HLA-A, -B, -DR, and -DQ was performed using standard serological methods (Danilovs et al. 1980). For the C4A and C4B determinations, the plasma samples were treated with carboxypeptidase B according to the procedure of Sim and Cross (1986) and then with neuraminidase according to the procedure of Awdeh and Alper (1980), followed by dialysis (Budowle 1983). Gel electrophoresis and development used the method of Awdeh and Alper (1980). Coagulation factor XIIIA (F13A1) was typed on the plasma samples by using agarose electrophoresis and visualization by fluorescence of monodansyl cadavarine (Graham et al. 1984). Southern analysis was also performed using the F13A1 cDNA probe and *Bgl*I-digested DNA, according to previously published protocols (Zoghbi et al. 1989). Glyoxalase (GLO1) genotypes were determined on hemolysates according to the method of Wraxall and Stolorow (1986). Genotypic analysis for the dinucleotide repeat polymorphism at the D6S89 locus was performed using PCR. DNA amplification was performed using primers 1717 and 1718, which flank the dinucleotide repeat tract at this locus (Litt and Luty 1990). The PCR was carried out in a total volume of 25 µl, using 40 ng of genomic DNA, 25 pmol of each primer, 1.25 mM $MgCl_2$, 250 µM dNTPs, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 0.01% gelatin, and 0.625 units Amplitaq polymerase. The 1717 primer was end labeled with γ-^{32}P dATP. For amplification, samples were denatured for 5 min at 94°C and were subjected to 25 cycles consisting of 2 min denaturation at 94°C, 1 min annealing at 55°C, and 2 min extension at 72°C, with a final extension step of 12 min at 72°C. A 4-µl sample of each reaction was fractionated by electrophoresis on a 4% polyacrylamide 7.65 M urea gel for 2.75 h at 1,100 V. Gels were exposed to Kodak XRP film for 20 h at −70°C.

The computer program SIMLINK (Ploughman and Boehnke 1989) was used to determine the probability of detecting linkage, given the pedigree structure and disease status of members. Marker data for F13A1, D6S89, and HLA/C4A/C4B were simulated in order to calculate the probability of a lod score greater than 3 and also to determine expected exclusion regions if the disease locus is not linked to any of these markers. Linkage analyses were performed using the LINKAGE package of computer programs (Lathrop et al. 1984). The variable age at onset of the SCA phenotype was taken into account by assigning asymptomatic individuals to age-dependent liability classes. Penetrance was assumed to increase linearly from 0 at age 15 years to 1.0 at age 40 years. Sex-specific estimates of the recombination fraction and the corresponding lod score were calculated between SCA1 and each of the chromosome 6 markers. The lod scores for various locations of the disease locus on a fixed map of these markers were also calculated. The male and female estimates of the recombination fractions for F13A1-D6S89 and D6S89-HLA/C4A/C4B were based on analyses of the CEPH data (Zoghbi et al. 1991). The Haldane mapping function was used to convert recombination fractions to map distances in centimorgans.

A multiple pairwise analysis using the MAP program (Morton and Andrews 1989) was also performed. The lod scores for HLA/F13A1 were taken from Keats et al. (1989), and those for D6S89/HLA and D6S89/F13A1 were based on CEPH data. This analysis used the mapping function of Rao et al. (1977), which includes an interference parameter, p; p may be set to any value, with $p = 1$ corresponding to no interference and with $p = 0$ corresponding to complete interference.

Results

The pedigree drawing in figure 1 shows all individuals included in the linkage analysis. Twenty-two individuals in this kindred have been diagnosed as affected, and 11 of these have received extensive neu-

rological examination. Seven of those affected are females, and 15 are males. All patients initially presented with the typical clinical picture of progressive gait and limb ataxia, followed by slurred speech. Within 10 years the disease progressed to include hyperreflexia, dysphagia, impaired vibration sense, distal weakness, and spasticity. These clinical features are very similar to those observed in other SCA1 kindreds (Nino et al. 1980; Haines et al. 1984; Zoghbi et al. 1988). Age at onset ranged from 15 to 35 years, with the mean $\pm$ standard error being 28.2 $\pm$ 1.7 in the fourth generation and 21.9 $\pm$ 1.5 in the fifth generation. At present over 30 offspring of affected individuals are at risk for ataxia. Although three individuals in this kindred did have relatively early ages at onset (15, 17, and 18 years), the majority of the patients had onset after 20 years of age. None of the patients had onset in early childhood as in the family described by Zoghbi et al. (1988).

Seven different alleles were identified at the D6S89 locus in this pedigree, and the 183-bp fragment (allele 14) segregated with the disease gene. In figure 1, D6S89 genotypes are given below each member from whom a blood sample was obtained. The correspondences between fragment lengths and allele designation in figure 1 are as follows: 2 = 207 bp; 3 = 205 bp; 5 = 201 bp; 9 = 193 bp; 11 = 189 bp; 13 = 185 bp; and 14 = 183 bp.

The results of the simulation studies indicated that for this kindred the probability of obtaining a lod score greater than 3 with D6S89 and HLA/C4A/C4B is at least .84, given that the disease locus is between these two markers. Alternatively, if the disease locus is unlinked to D6S89 and HLA/C4A/C4B, then the probability of a lod score less than -2 at any location between these two markers is greater than .92. The simulation studies also showed that the probability of negative lod scores at all locations between F13A1 and HLA/C4A/C4B is less than .06, given that they flank the disease locus.

Pairwise lod scores between SCA1 and each of the chromosome 6 markers were calculated for the sexes pooled, as were sex-specific estimates of the recombination fractions (table 1). No recombination events were observed among the HLA, C4A, and C4B loci, so haplotypes were constructed and used in the linkage analyses. The results for HLA/C4A/C4B exclude the SCA1 locus from a distance of approximately 6 cM on either side of the HLA region, and the maximum likelihood estimate of the recombination fraction is .5, suggesting that the disease locus in this family may not be linked to HLA. Similarly, the lod scores between SCA1 and F13A1 suggest that these two loci are not linked. A three-point analysis of HLA/C4A/C4B, F13A1, and the disease locus confirms this exclusion region (fig. 2), and the lod score is below -1.4 at all points between F13A1 and HLA. However, the maximum lod score of 4.90 at a recombination fraction of 0, both in males and in females, between the disease locus and D6S89 (table 1), shows that the disease locus in this family is on the short arm of chromosome 6.

In order to determine the most likely location of the SCA1 locus in this family, lod scores for various positions on the genetic map were calculated. The male locations of F13A1, D6S89, and HLA/C4A/C4B were fixed at 0, 16, and 33 cM, respectively, with

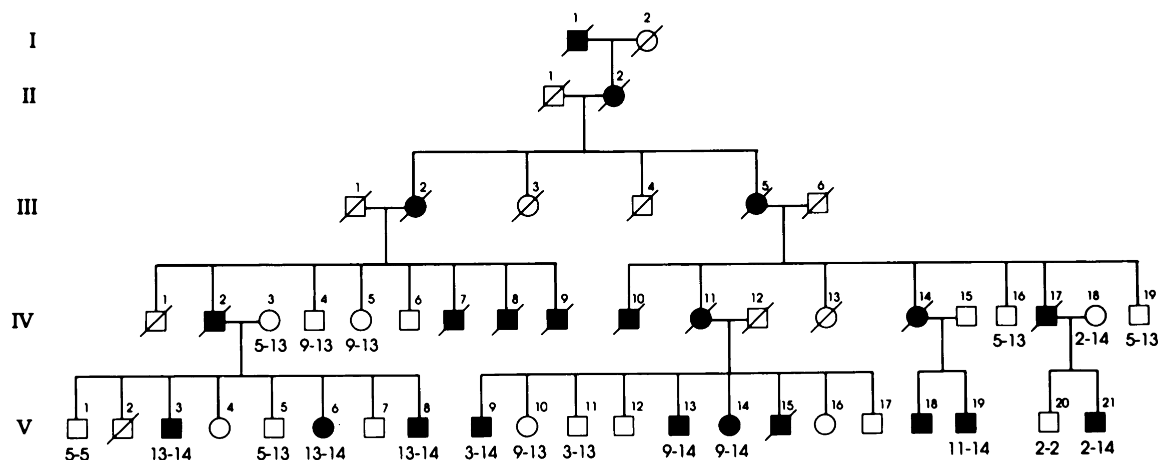

Figure 1 Condensed pedigree of family with autosomal dominant spinocerebellar ataxia showing D6S89 genotypes

Table 1

Pairwise Linkage Results for SCA1 with 6p Markers

Marker	Maximum Lod Score	Maximum Recombination Fraction	Lod Score at Recombination Fraction of							Maximum Male Recombination Fraction	Maximum Female Recombination Fraction	Maximum Sex-specific Lod Score
			.001	.01	.05	.1	.2	.3	.4			
F13A1	.10	.25	−4.46	−2.41	−.91	−.31	.07	.09	.02	.50	.21	.19
D6S89	4.90	.00	4.89	4.80	4.40	3.89	2.83	1.74	.70	.00	.00	4.90
HLA/C4A/C4B ...	.0	.50	−5.48	−3.50	−2.15	−1.52	−.82	−.44	−.18	.50	.50	.0
GLO1	.0	.50	−6.90	−3.90	−1.84	−1.01	−.35	−.10	−.03	.50	.50	.0

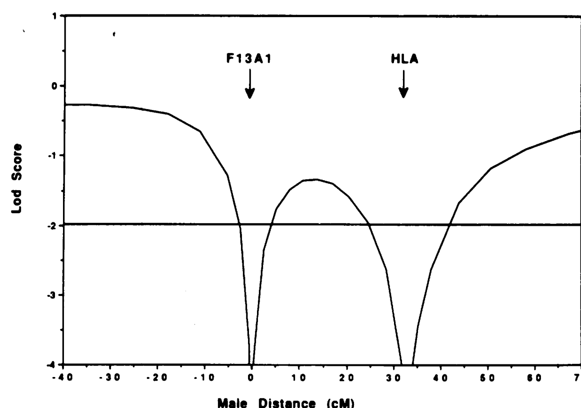

Figure 2 Localization of SCA1 locus on fixed map consisting of F13A1 and HLA.

the corresponding female locations being 0, 19, and 57 cM; the results are plotted in figure 3. An age-at-onset correction was included in this analysis, but the results were almost the same if all asymptomatic individuals were assumed not to have the disease gene. The maximum score occurs at the location of D6S89, but the SCA1 locus may be as much as 10 cM either proximal or distal to D6S89. However, a location outside the F13A1-HLA interval is excluded.

The multiple pairwise approach allows for interference, and in this analysis the parameter p was set to a value of .35. Fixing the map of F13A1, D6S89, and HLA/C4A/C4B gave similar results to those shown in figure 3 for various locations of SCA1. Further analyses were performed to construct maps by using lod score data and recombination estimates for all six pairwise comparisons of the four loci. The best map placed SCA1 very close to D6S89, between F13A1 and HLA,

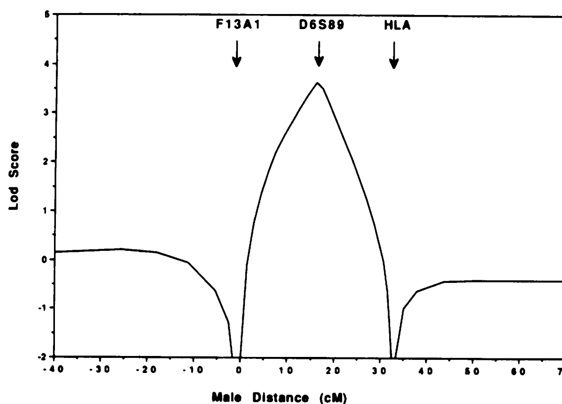

Figure 3 Localization of SCA1 locus on fixed map consisting of F13A1, D6S89, and HLA.

and the total lod score was at least 3 greater than that for a location outside the F13A1-HLA region.

Discussion

In initial analyses of linkage to HLA, the gene causing SCA in this American black family was excluded from at least 6 cM on either side of HLA, and the maximum lod score was 0 at a recombination fraction of .5, suggesting that the disease locus may not be in this region. Similarly, close linkage to F13A1 was excluded, and the maximum lod score for locations between HLA and F13A1 was − 1.4. This suggestive evidence of exclusion was reinforced by the simulation studies, which showed that the probability of obtaining a negative lod score at all locations between HLA and F13A1, if the disease locus is in this region, is less than .06. However, further studies using a dinucleotide repeat polymorphism at the D6S89 locus, which is distal to the HLA loci (17 cM in males and 38 cM in females), showed tight linkage between the disease locus and D6S89, confirming that this family indeed segregates for SCA1, the locus on the short arm of chromosome 6. Multipoint linkage analysis using the F13A1, D6S89, and HLA/C4A/C4B loci confirmed that SCA1 is near the D6S89 locus, but whether the disease locus is proximal or distal to D6S89 could not be inferred. This analysis strongly supports a location distal to HLA and proximal to F13A1.

Recent studies of several kindreds with the HLA-linked form of SCA have shown that in each family the SCA1 locus is closely linked to D6S89 (Ranum et al. 1991; Zoghbi et al. 1991). It is noteworthy, however, that the family studied by Zoghbi et al. (1989) failed to show linkage between the disease locus and F13A1 and that, in fact, prior to the availability of the D6S89 data, the location of the gene causing SCA in this family was thought to be centromeric to the HLA region. Analysis of the dinucleotide repeat polymorphism at D6S89 resolved the ambiguity regarding the precise position of SCA1 on 6p. On the basis of these data and the results from the present study, it is clear that families with SCA for which close linkage to HLA has been excluded should be typed for D6S89, in order to determine whether the disease locus is SCA1.

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