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Assignment of Autosomal Dominant Spinocerebellar Ataxia (SCA1) Centromeric to the HLA Region on the Short Arm of Chromosome 6, Using Multilocus Linkage Analysis

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Summary

A 7-generation kindred with the HLA-linked form of spinocerebellar ataxia (SCA1) was studied to determine whether the SCA1 gene maps centromeric or telomeric to the HLA loci. The DNA markers flanking the HLA-(A-B) region were used for polymorphism studies and multilocus linkage analysis. These two markers are the cDNA for the β-subunit of HLA-DP, which is centromeric to HLA-(A-B), and the cDNA for coagulation factor XIIIa (F13A), which is telomeric to HLA-(A-B). Haplotypes were constructed using multiple polymorphisms for these two DNA markers, and pairwise linkage analysis revealed a maximum lod score of 2.18 for SCA1 versus HLA-DP at a recombination fraction of .05 and a maximum lod score of 0 for SCA1 versus F13A at a recombination fraction of .50. A possible crossover between HLA-(A-B) and HLA-DP was identified, but lack of samples from key individuals hampered the analysis. To clarify the phase and improve the analysis, the two chromosomes 6 for the crossover individual were separated in somatic cell hybrids. The results strongly favored the probability that the crossover occurred between HLA-(A-B-DR) and HLA-DP with SCA1 segregating with HLA-DP, consistent with a location centromeric to HLA-(A-B). Multilocus linkage analysis was used to evaluate further the location of SCA1 relative to F13A, HLA-(A-B), and HLA-DP; the results indicated that the SCA1 gene locus is centromeric to HLA-DP with odds of 46:1 favoring this most likely location over the second most likely location, i.e., telomeric to HLA-(A-B) between the HLA complex and F13A.

Introduction

The dominantly inherited spinocerebellar ataxias (SCA) are a group of neurodegenerative disorders characterized by neuronal loss in the cerebellum with variable involvement of the brain stem and spinal cord (Koeppen and Barron 1984). Affected individuals suffer from various combinations of progressive ataxia, motor disturbance, peripheral neuropathy, and intellectual deterioration (Schut 1954; Currier et al. 1972; Nino et al. 1980; Berciano 1982). The onset of symptoms is usually delayed until the third to fifth decade (Currier et al. 1972; Nino et al. 1980; Haines et al. 1984), although onset as early as 4 years or as late as the seventh decade has been documented (Zoghbi et al. 1988*b*). The disease progresses, resulting in complete disability and, ultimately, in death 10–15 years after onset.

SCA1 is one subtype of the dominantly inherited ataxias, with its gene being assigned to the short arm of chromosome 6 near the human leukocyte antigen (HLA) locus (Lamm and Olaisen 1985). Attempts at mapping the precise location of the SCA1 gene locus with respect to HLA and other 6p markers have been limited owing to the lack of highly informative markers on 6p outside of the HLA region (Haines and Trofatter 1986; Rich et al. 1987). We have studied a 7-generation kindred with SCA1. Elsewhere, linkage to HLA-(A-B)

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was confirmed in this kindred with a maximum lod score of 5.83 at a recombination fraction of .12 (Zoghbi et al. 1988*b*). Several polymorphisms have been identified using the cDNA for coagulation factor XIIIa (Board et al. 1988; Zoghbi et al. 1988*a*). In this report we have used the polymorphisms for factor XIIIa and the cDNA clone for the β-subunit of HLA-DP to perform multilocus linkage analysis for SCA1 in order to map the location of the SCA1 gene relative to these markers. In our initial evaluations, it appeared that this family would not provide sufficient information to resolve completely our questions regarding gene order. We therefore isolated three patients' chromosomes 6 in somatic cell hybrids to gather further phase information for multilocus analysis.

Material and Methods

Subjects

The kindred under study is of American black ancestry with occasional known Caucasian ancestors. Data on clinical information, cumulative onset probability, HLA typing, and linkage relationships to HLA-A, B, DR, and DQ have been reported elsewhere (Zoghbi et al. 1988*b*). Southern blotting analyses with the different DNA markers were carried out on at least 52 individuals from this kindred.

Molecular Studies

Two DNA probes flanking the HLA-(A-B) region were utilized. The first was the cDNA probe for the β-subunit of HLA-DP (DPβ), which was kindly provided to us by Bo Servenius (Research Institute of Scripps Clinic, La Jolla, CA). DPβ maps centromeric to HLA-(A-B) at an estimated genetic distance of 3–4 centimorgans (cM) (Lamm and Olaisen 1985). The DNA polymorphisms detected by the DPβ probe have been described by Hyldig-Nielsen et al. (1987). The second probe was the cDNA probe for coagulation factor XIIIa (gene symbol F13A), which was kindly provided to us by Ulrich Grundmann (Behringwerke AG, Marburg, West Germany). Elsewhere F13A has been mapped telomeric to HLA-(A-B) at a recombination fraction of .19 in males, as a result of linkage studies using protein polymorphisms at that locus (Lamm and Olaisen 1985). We used an 1,161-bp fragment from the 5′ end of the cDNA for F13A and identified several RFLPs (Zoghbi et al. 1988*a*). Two polymorphisms detected with the restriction enzyme *Bam*HI and one multiallelic RFLP detected with *Bcl*I were used for studies of linkage relationships to the HLA-(A-B-DR) loci in the reference families provided by the Centre d'Etude du Polymorphisme Humain (CEPH) (Zoghbi et al. 1988*a*). F13A was found to be 43 cM (recombination fraction .29) from the HLA-(A-B-DR) loci in the CEPH families (confidence interval 32–51 cM). To study linkage relationships between these two probes and SCA1, the restriction enzyme *Msp*I was used for the DPβ probe, and the restriction enzymes *Bam*HI, *Bcl*I and *Fnu*4HI were used for the F13A probe.

DNA for Southern transfer analysis was isolated from blood samples or cultured lymphoblastoid cells as described elsewhere (Zoghbi et al. 1988*a*). Southern blotting analysis using the DPβ and F13A cDNA probes were performed as described elsewhere (Zoghbi et al. 1988*a*).

Somatic Cell Hybrids

Somatic cell hybrids were used to separate the maternal and paternal chromosomes 6 of one individual who carries a possible recombination within the HLA region. On the basis of results to be shown below, separating the two chromosomes in a somatic cell hybrid established the phase for HLA-DP, SCA1, and F13A.

To prepare the somatic cell hybrids, the cell line GB, a lymphoblastoid cell line transformed with Epstein-Barr virus, was used. This cell line is from a male affected with SCA1 and has a possible recombination within the HLA complex. GB cells were fused to RJK88 cells, a derivation of the Chinese hamster lung cell line V79, which, owing to a gene deletion, is deficient in hypoxanthine phosphoribosyl transferase (Fuscoe et al. 1983).

Fusions were performed in monolayers by using modifications of a standard technique (Nussbaum et al. 1983; R. L. Nussbaum, personal communication). Two 100-mm plates were seeded with 10^7 RJK88 cells/plate 16–20 h before the fusion. The plates containing RJK88 were washed with Dulbecco's modified Eagle medium (DMEM) (Hazelton, Lenexa, KS), and the medium was then aspirated completely. Then 250 μl of a 1-mg stock of phytohemagglutin-P/ml (Sigma, St. Louis) was added to a 10-ml suspension of 5×10^7 lymphoblasts in Hanks balanced saline (Gibco, Grand Island, NY). Five milliliters of lymphoblasts (2.5×10^7 cells) was added to each plate containing the RJK88 cells, and the plates were incubated for 15 min at 37°C. The Hanks solution was then gently aspirated, leaving behind many lymphoblasts adherent to the rodent cells. Two milliliters of 50% polyethylene glycol 1500 (PEG; Boehringer Mannheim Biochemicals, Indianapolis) was added and spread over the surface of the plate. After 1 min, the PEG was aspirated, and the

plate was rinsed three times with DMEM and incubated for 30 min at 37°C. After incubation, the medium was aspirated, 10 ml of DMEM with 10% FCS was added, and the plates were incubated overnight. Hybrids were selected for by growth in DMEM containing 10% FCS, 0.1 mM hypoxanthine, and 50 μM azaserine. Surviving colonies were isolated with cloning rings 2 wk later and were grown for DNA preparation. The retention of either the maternal or paternal chromosomes 6 was evaluated by Southern blotting analysis using *Bam*HI- and *Bcl*I-digested DNA with F13A as probe and by using *Msp*I-digested DNA with DPβ as a probe. Somatic cell hybrids were also prepared using the lymphoblast cell lines from individuals V-8 and V-9 to separate their maternal and paternal chromosomes 6. The HLA-(A-B-DR) haplotypes for their mother, individual IV-5, were determined prior to her death. Accordingly, the maternal and paternal chromosomes 6 for individuals V-8 and V-9 were identified using HLA-A, HLA-B, and HLA-DR probes.

Linkage Analysis

Pairwise and multilocus linkage analyses were performed using the LINKAGE package of computer programs (Lathrop et al. 1984). Genetic distances were calculated using Haldane's mapping function, which converts recombination frequencies to genetic distances in centimorgans.

In some areas of the human genome, a difference in recombination frequency between males and females has been observed. In our previous study of F13A and HLA-(A-B-DR), we detected a marginally significant difference between male and female recombination frequencies on the short arm of chromosome 6, the ratio between the genetic distance in females as compared with males being 1.75:1. Because of this difference, although it was marginally significant, all analyses were carried out both under the assumption of equal recombination frequencies and under the assumption of a 1.75:1 ratio between female and male genetic distances. In multilocus linkage analysis, likelihoods were calculated for several locations of the disease gene relative to a map of markers. For each location, the likelihood can be compared with that of the observed data when the disease gene is unlinked to the map of markers (resulting in a lod score), or the likelihood can be compared with that obtained for another location (resulting in odds favoring one location over another).

Because the expression of the SCA phenotype is age dependent, the probable genotypes for asymptomatic individuals were calculated using a stepwise age-at-onset function described elsewhere (Zoghbi et al. 1988*b*). Allele frequencies and haplotype frequencies for HLA-A, B, and A-B, were obtained from the report of the Eighth International Histocompatibility Workshop (Baur and Danilovs 1980). Since there are no established allele frequencies for HLA-DP and F13A in American blacks, allele frequencies were calculated using genotypic data of 28 independent chromosomes for HLA-DP and genotypic data of 34 independent chromosomes for F13A from individuals marrying into the SCA1 family. The recombination frequency between HLA-B and HLA-DP was fixed at .04 (.04 for males in the analysis with sex-specific recombination frequencies) (Lamm and Olaisen 1985). The recombination frequency between HLA-A and F13A was fixed at .29 (.25 in males and .35 in females, when allowing for sex difference) (Zoghbi et al. 1988*a*). No recombinations were observed between HLA-A and HLA-B; hence, in multilocus analysis these two loci were regarded as one locus by using population haplotype frequencies for HLA-(A-B) as allele frequencies for this combined locus. To increase computational efficiency, marker alleles were recoded according to the methods of Ott (1978) and Braverman (1985). For all recoded markers, comparisons were made between lod scores obtained with original and with recoded marker data in two-locus analysis of disease versus marker. All comparisons yielded identical results. For a subset of the family (individuals III-4 and III-5 and their descendants in the original kindred described by Zoghbi et al. [1988*b*]), data for four independent polymorphisms at the F13A locus were recorded as haplotypes. In the final analysis, results for two three-locus analyses both using data for HLA-(A-B) and SCA were combined. In one analysis the marker HLA-DP being centromeric to HLA-(A-B) was added; in this analysis we only evaluated location of SCA1 centromeric to HLA-(A-B). In the second analysis, the data for F13A were used, and in this analysis only locations telomeric to HLA-(A-B) were evaluated. Finally lod scores obtained in both analyses were compared directly.

Results

Polymorphism Data

Six different polymorphic DNA fragments were detected using *Msp*I-digested DNA and the DPβ probe. Figure 1 shows the polymorphic DNA fragments in family members from the Houston SCA1 kindred. Haplotypes were constructed using these DNA fragments, as shown in figure 1. The individuals shown were chosen

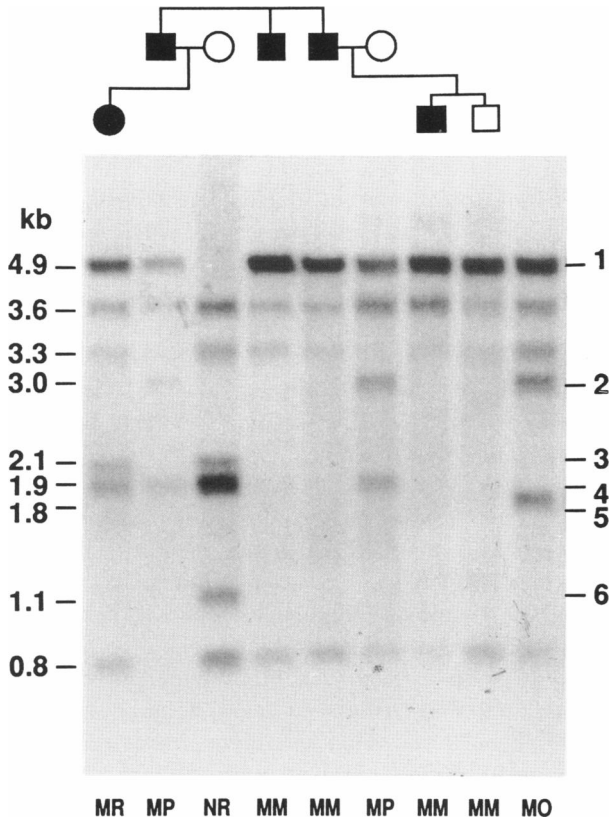

Figure 1 RFLPs detected with *Msp*I and the DBβ probe. The six variable bands are marked on the right. The pedigree at the top illustrates the relationship of the individuals in lanes 1–8. Lane 9 is from an unrelated individual. Haplotypes M (band 1), N (bands 4 and 6), O (bands 2 and 5), P (bands 2 and 4), and R (bands 3 and 4) are shown at the bottom of the figure.

because their genotypes display five different haplotypes observed in this kindred. Two *Bam*HI polymorphisms, the *Bcl*I polymorphism, and the *Fnu*4HI polymorphism were used for pairwise linkage analysis between SCA1 and F13A and for the multilocus linkage analysis between F13A, HLA-(A-B), HLA-DP, and SCA1.

Somatic Cell Hybrid Analysis and Analysis of an HLA-SCA1 Recombinant

For an individual who is heterozygous at more than one locus, it is essential to establish which alleles for both loci are on the same chromosome, in order to determine whether recombinations occurred in that person or in the offspring. This detailed description of the person's genotype is often considered as establishing the person's phase for the loci. For two loci, two different possibilities exist; for three loci there are four possibilities. Occasionally, this information can be deduced with absolute certainty from the genotypes of the parents, but in many cases, where no absolute certainty exists, all possible phases have to be evaluated with the LINKAGE computer programs. However, each person contributes the maximum amount of information for linkage analysis only when phase is known with complete certainty. For one individual in the SCA family who might be carrying a highly informative recombination, phase could not be established with certainty. Therefore, we applied somatic cell hybridization techniques, to analyze each individual chromosome 6 of that person.

Several somatic cell hybrid clones were isolated after fusion of GB lymphoblasts to RJK88 cells. Although no selection for retention of chromosome 6 was used, seven of 26 independent clones retained chromosome 6, as demonstrated by DNA analysis. Of these seven, five clones retained the maternal chromosome 6 (as identified by its haplotype for one of the markers, e.g., F13A), one retained the paternal chromosome 6, and one retained both. Southern blotting analysis using *Msp*I-digested DNA and the DPβ cDNA probe shows the maternal haplotype in hybrid cell line (GB-M) and the paternal haplotype in hybrid cell line (GB-P) for HLA-DP in figure 2*A*. Figures 2*B* and 2*C*, respectively, show the maternal *Bam*HI and *Bcl*I alleles in hybrid cell line (GB-M) when the F13A cDNA probe was used; the paternal alleles are also shown in hybrid cell line GB-P. cDNA for the β-subunit of HLA-DR was also used with *Taq*I-digested DNA to determine the maternal and paternal HLA-DR haplotypes (data not shown). The maternal and paternal chromosomes 6 were identified similarly for individuals V-8 and V-9 (data not shown).

Figure 3 shows a small branch from the Houston SCA1 pedigree. GB is individual VI-13 in this reduced pedigree and in the detailed pedigree reported by Zoghbi et al. (1988*b*). Haplotype data (coded by letters) are shown for F13A, HLA-(A-B-DR), and HLA-DP. The SCA1 status, affected or normal, is also shown. Individual IV-5 had undergone HLA typing prior to her death. The HLA data for individual IV-6 and the F13A and HLA-DP data for individuals IV-5 and IV-6 were absolutely implied on the basis of the knowledge of the genotypes and of the phase for the maternal and paternal chromosomes 6 in their daughters, V-8 and V-9. With regard to the HLA-(A-B-DR) data, individual IV-5 was affected and has inherited the SCA1 gene from her affected father, who also contributed the HLA

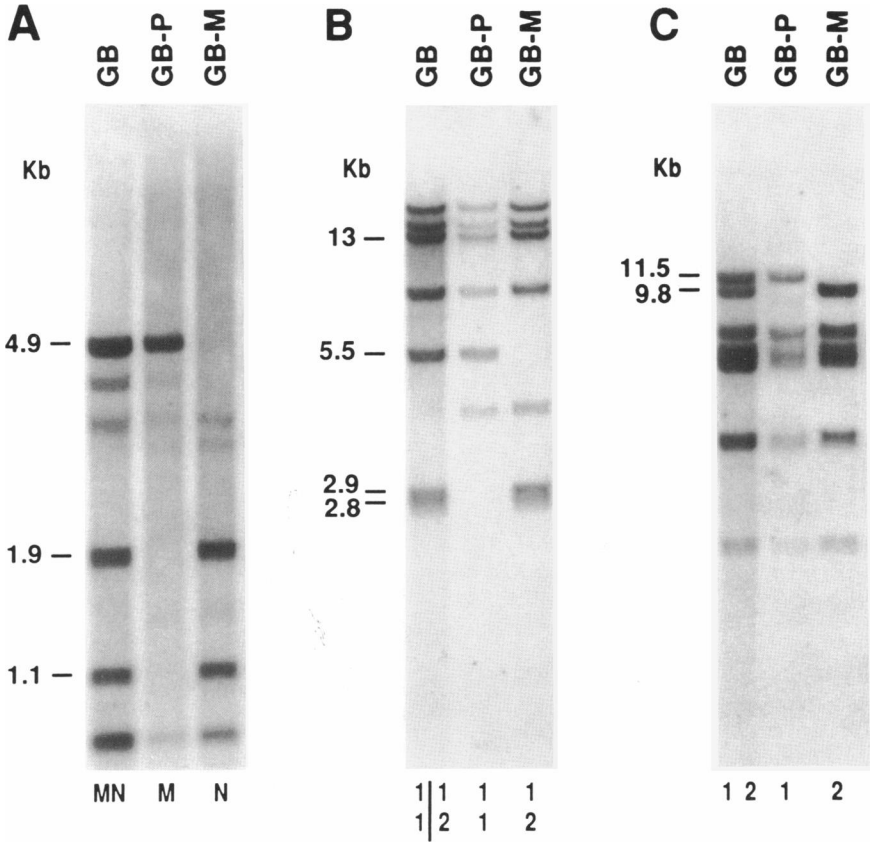

Figure 2 Southern blotting analysis of the somatic cell hybrids derived from the fusion of the cell line GB with Chinese hamster cells. Panel *A* shows *Msp*I-digested DNA from the parent cell line GB and the hybrids retaining either paternal chromosome 6 (GB-P) or maternal chromosome 6 (GB-M); all samples were probed with DPβ. The 4.9-kb fragments mark the maternal chromosome. Panel *B* shows a *Bam*HI digest of the same cell lines probed with F13A; the 13-kb fragment marks the first polymorphism (genotype 1,1), and the 5.5-kb, and 2.9- and 2.8-kb fragments mark the second *Bam*HI polymorphism (genotype 1,2). Haplotypes for these two polymorphisms are denoted at the bottom of the figure. Panel *C* shows a *Bcl*I digest of these cell lines probed with F13A. The 11.5-kb fragment (allele 1) marks the paternal chromosome, and the 9.6-kb fragment (allele 2) marks the maternal chromosome. The bands at approximately 4 kb in the GB-M and GB-P lanes are of hamster origin.

haplotype G. The G haplotype appears with SCA1 in the majority of the descendants of III-5 and III-10 in the larger Houston SCA1 pedigree. In this reduced pedigree, the G haplotype was associated with disease in IV-5 and V-8 and may have been present in V-6. Two recombinations between HLA-(A-B-DR) and SCA1 were identified in individuals V-9 and VI-13. In individual V-9, the recombination occurred in the maternal chromosome, but it was not possible to determine whether this recombination occurred within the HLA cluster. To determine whether the crossover in individual VI-13 was within the HLA complex, between HLA-(A-B-DR) and HLA-DP, it was important to determine the origin of both HLA-DP alleles in this individual. This was done as follows: One starts with the knowledge that there are three different F13A haplotypes (A, B, and C) among individuals IV-5 and IV-6 in figure 3. As VI-13 must have inherited one of these haplotypes through his mother, the B haplotype must be maternally derived —rather than the D haplotype, which was not seen in higher generations. Similarly, for HLA-(A-B-DR), the haplotypes G, H, I, and J are the haplotypes seen in the maternal grandparents; hence, the haplotype I was maternally derived and haplotype K was paternally derived. Southern analysis of the two chromosomes 6 of GB (VI-13) separated in somatic cell hybrids permitted the unambiguous assignment of the N haplotype for HLA-DP to the maternally derived chromosome. This

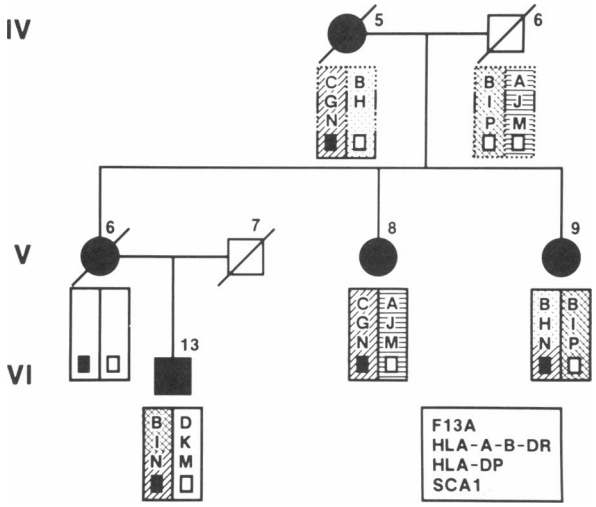

Figure 3 Genetic analysis for F13A, HLA-(A-B-DR), and HLA-DP in a subset of the family in whom a possible recombinant is identified within the HLA region in VI-13 (cell line GB). Data for the three markers are presented as haplotypes; F13A haplotypes are indicated as A–D, HLA-(A-B-DR) as G–K, and HLA-DP as M, N, or P. SCA1 status is indicated by a closed rectangle for affected and by an open rectangle for normal. Solid lines indicate definitively known phase (which for individuals V-8, V-9, and VI-13 was determined using somatic hybridization techniques), and dotted lines indicate that the phase was unknown but that the assignment is the most probable on the basis of the available data. The G haplotype for HLA-(A-B-DR) in individual IV-5 was inherited from her affected father and identifies the chromosome carrying the SCA1 mutation. Two recombinants are identified in individuals V-9 and VI-13. It is not possible to determine whether the recombination in individual V-9 is within the HLA cluster. The recombination in individual VI-13 occurred within the HLA cluster between HLA-(A-B-DR) and HLA-DP, as is explained in the text.

means that GB inherited a recombinant chromosome from his mother, receiving the I haplotype for HLA-(A-B-DR) from the maternal grandfather and the HLA-DP haplotype from the maternal grandmother. These data suggest that the SCA1 locus is centromeric to the HLA cluster.

Genetic Linkage Data

Two-locus linkage analysis.—Two-locus lod scores were calculated at several recombination fractions for F13A, HLA-(A-B), HLA-DP, and the disease locus (table 1). At least one recombination was observed between SCA1 and each marker tested. A maximum lod score of 2.77 was obtained for SCA1 versus HLA-A at a recombination fraction of .15, and a significant lod score of 4.06 was obtained for SCA1 versus HLA-B at a recombination fraction of .11. The maximum lod score for HLA-DP versus SCA1 was 2.18 at a recombination fraction of .05. Linkage between SCA1 and F13A was excluded (lod score ≤ -2) at a distance of at least 10 cM. The two-locus linkage analysis suggests a map location of SCA1 centromeric to HLA.

Multilocus Linkage Analysis.— Multilocus lod score calculations using all marker and disease data simultaneously require unmanageably large amounts of computing time in the case of this family. This is due to individuals in the older generations, for whom marker results cannot be obtained. Accordingly, we applied comprehensive multilocus analysis to the main part of the pedigree only, including individuals III-4 and III-5 and descendants. For all deceased persons in this part of the pedigree, marker genotypes could be inferred.

Figure 4 shows the lod scores for multilocus linkage analysis of SCA1, tested against a fixed map of the three polymorphic loci on the short arm of chromosome 6. The overall maximum lod score for SCA1 was obtained at approximately 5 cM (recombination fraction .05) centromeric to HLA-DP. The lod score at this location was 3.21, which represents a 1,600:1 odds in favor of this location when compared with a location unlinked to HLA-DP or HLA-(A-B). The maximum lod score for the interval between HLA-(A-B) and F13A was 1.54 (34:1 odds) with a recombination fraction between HLA-(A-B) and SCA1 of .09. For locations distal to F13A, and between HLA-(A-B) and HLA-DP, only nega-

Table 1

Pairwise Linkage Results for SCA1 and 6p Marker Loci

Loci	Recombination Fraction						Maximum		95% Confidence Interval
	0	.05	.1	.2	.3	.4	Lod Score	Recombination Fraction	
SCA1:F13A	$-\infty$	−4.88	−2.94	−1.25	−.49	−.13	0	.5	. . .
SCA1:HLA-A	$-\infty$	1.75	2.58	2.68	2.07	1.13	2.77	.15	.05–.34
SCA1:HLA-B	$-\infty$	3.60	4.04	3.66	2.67	1.41	4.06	.11	.03–.27
SCA1:HAL-(A-B)	$-\infty$	2.74	3.50	3.42	2.57	1.35	3.62	.14	.05–.29
SCA1:HLA-DP	$-\infty$	2.18	2.07	1.56	.98	.44	2.18	.05	.001–.26

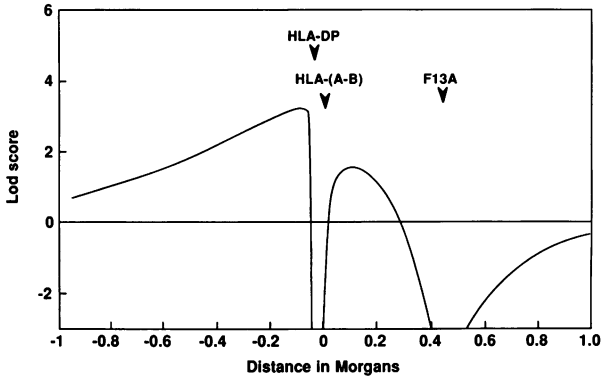

Figure 4 Two separate lod score curves on each side of HLA-(A-B) from two different three-point linkage analyses. For each linkage analysis lod scores were calculated as log base 10 of (likelihood for specific location divided by likelihood obtained when disease gene is unlinked to the same group of markers).

tive lod scores were obtained. Comparison of the lod score results of these analyses provides strong evidence that SCA1 is located on the centromeric side of HLA. Odds favoring a centromeric location versus the second most likely location—i.e., between HLA-(A-B) and F13A—are 46:1. All analyses were also carried out assuming a higher frequency of recombination in females than in males. Results were consistent with the results assuming equal recombination rates. Odds favoring centromeric location are somewhat lower (27:1), although still strongly in favor of that location. By contrast, in analyses in which the results of somatic cell hybrids in one key person were ignored, the odds favoring centromeric location were only 6.5:1 (maximum lod score of 2.35 for a location at 9 cM centromeric of HLA-DP). In summary, multilocus linkage analysis is consistent with the results of two-locus analysis and provides strong evidence for a centromeric location for SCA1.

Discussion

SCA is a devastating neurodegenerative disorder. Most individuals with SCA1 are diagnosed past the reproductive age, and each offspring of an affected individual is at 50% risk for having inherited the defective gene. This creates a need to develop an accurate presymptomatic test for the disease. Availability of very tightly linked DNA markers for SCA1 would provide such a diagnostic tool.

Linkage studies to the HLA loci have mapped the SCA1 gene at a genetic distance of 12–15 cM from either side of HLA-(A-B) (Yakura et al. 1974; Jackson et al. 1977; Nino et al. 1980; Pedersen et al. 1980; Haines et al. 1984; Zoghbi et al. 1988*b*). In an attempt to map the SCA1 gene locus more precisely on 6p, we chose to use HLA-(A-B) and two very highly informative markers flanking HLA-(A-B), for analysis of recombinants and multilocus linkage analyses. The two markers used were the β-subunit for HLA-DP and F13A; both markers have an established genetic distance from HLA-(A-B).

On inspection of this pedigree, it is clear that several meioses are informative for all markers tested. Several recombinations that provide important information to establish gene order are present. One possible recombination is the one within HLA, between (A-B-DR) and DP in individual VI-13. Analysis of this possible recombination was made feasible through the use of somatic cell hybrids to establish the phase for both HLA-DP and the other markers in that individual. The results indicated that a recombination most probably occurred within the HLA complex and that SCA1 segregated with HLA-DP. These findings suggest that SCA1 maps centromeric to the HLA complex. To quantitatively assess the probability that a centromeric location for SCA1 was most likely, we performed multilocus linkage analysis. In our multilocus linkage analysis, we focused on a subset of the pedigree in which marker data were available or could be deduced for all family members. We obtained results for two sets of three-locus analyses—one for HLA-(A-B), HLA-DP, and SCA1 and the other for HLA-(A-B), SCA1, and F13A—because it is technically not feasible to perform four-point linkage analysis, given the large number of haplotypes at the HLA and F13A loci. The results of a simultaneous four-point analysis should be similar to those presented here because the HLA-(A-B) locus is informative for all meioses in the subset of the family studied separately. No extra information regarding phase for HLA-(A-B) can therefore be provided by other neighboring markers. Therefore, additional data for a distal locus such as F13A will not provide new information for locations of SCA1 proximal of HLA-(A-B), and data for a proximal locus such as HLA-DP will not provide new information for locations of SCA1 distal to HLA-(A-B). Comparison of lod score results of the multilocus linkage analysis provides strong evidence (46:1 odds) that SCA1 is located on the centromeric side of HLA. Even when one allows for a higher frequency of recombination in females, results still consistently support a centromeric location of SCA1. It is important to note that these odds represent the most conservative odds because they were derived using a very conservative age-at-onset correc-

tion function. In our judgment the age-at-onset function underestimated the probability of manifesting the SCA phenotype at specific ages because of the wide variability in anecdotally collected data regarding the age at onset in deceased individuals. Accordingly, an analysis was done using an age-at-onset correction derived from data collected only on those individuals who were actually examined (i.e., all individuals from whom blood samples were drawn for linkage analysis). The results of the analysis using this age-at-onset correction yielded odds of 186:1 in favor of a centromeric location (data not presented).

The data presented are in disagreement with previous reports regarding the gene order of SCA1 with respect to HLA-(A-B). Haines and Trofatter (1986) performed multilocus linkage analysis using SCA1, HLA-A, HLA-B, properdin factor b, and glyoxalase (GLO). They found relatively low odds (7.2:1) in favor of positioning SCA1 telomeric to HLA-A. The authors acknowledged that these low odds are probably due to the relative lack of informative matings using GLO as a centromeric marker and suggested that other, more informative centromeric markers be used. In another study, Rich et al. (1987) used HLA-A and the anonymous genomic sequence 7H4 for multilocus linkage studies with SCA1. With these markers, the results indicated 18:1 odds favoring a telomeric location for SCA1 between HLA-A and 7H4. A major problem with the analysis was the decision of the authors to use a genetic distance for 7H4 that is quite different from the previously published data (Leach et al. 1986). The odds for telomeric location of SCA1 would most likely decrease considerably when 7H4 is located at a recombination fraction of .49 from HLA-(A-B), reported by Leach et al. (1986). It would be extremely useful to do multilocus linkage analysis in the kindred studied by Haines and Trofatter and by Rich et al., using HLA-DP and F13A as the additional 6p markers. It is possible that the Houston SCA1 kindred and the kindred studied by Haines and Trofatter and by Rich et al. have different mutations mapping to different regions on chromosome 6p. This would be quite interesting in view of some of the variability of the clinical presentation among the two kindreds. Analysis from the data set presented in the present study suggests that the most probable location of SCA1 is centromeric to the HLA loci.

Determining that the genetic location of SCA1 on 6p is centromeric to HLA provides a focus for identifying tightly linked DNA markers. The goal would be to isolate DNA markers that flank the SCA1 gene locus at 0–1 cM. Cell lines carrying 6p deletions or translocations and somatic cell hybrids would help one to identify DNA probes mapping centromeric to HLA and within detectable genetic distance of that locus. Such markers will delineate the starting point both for long-range physical mapping using pulsed-field gel electrophoresis and for chromosome walking. The ultimate goal would be to clone the gene for SCA1 and subsequently to study the biology of the normal and mutant genes. Until the SCA1 gene is cloned and characterized, tightly linked DNA markers will be extremely useful for presymptomatic diagnosis. Since such markers are not available yet, SCA family members at risk may choose to use HLA-(A-B) typing and HLA-DP DNA studies for presymptomatic diagnosis and prenatal testing.

Finally, results of this study clearly show that somatic cell hybrids can be used to resolve questions regarding phase in potentially highly informative individuals. Hybrids are being prepared for numerous individuals in the Houston SCA1 kindred. This approach will prove useful for all those diseases in which gene localization by simple linkage analysis in single large families is inconclusive.

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