Am. J. Hum. Genet. 49:31–41, 1991

Localization of the Autosomal Dominant HLA-linked Spinocerebellar Ataxia (SCA1) Locus, in Two Kindreds, within an 8-cM Subregion of Chromosome 6p

Laura P. W. Ranum,* Lisa A. Duvick,* Stephen S. Rich,* Lawrence J. Schut,† Michael Litt,‡ and Harry T. Orr*

*Department of Laboratory Medicine and Pathology, and Institute of Human Genetics, University of Minnesota, and †Veterans' Administration Medical Center, Minneapolis; and ‡Departments of Biochemistry and Medical Genetics, Oregon Health Science University, Portland

Summary

Two large kindreds with HLA-linked, autosomal dominant spinocerebellar ataxia (SCA1) were examined with markers from chromosome 6p to determine the location of the SCA1 locus. Results of the three-point analysis between the markers HLA-A, SCA1, and F13A overwhelmingly favor the conclusion that SCA1 is located distal of HLA and proximal of F13A. In addition, our data strongly support the conclusion that SCA1 lies centromeric and genetically very close to the highly informative D6S89 marker within the 8-cM chromosomal segment flanked by the D6S88 and D6S89 markers. In the two kindreds, one recombinant was observed between D6S89 and SCA1, resulting in a recombination fraction of .014 between the two loci.

Introduction

The inherited spinocerebellar ataxias are progressive neurological disorders characterized by symptoms and signs of degeneration of the cerebellum, spinal cord, and brain stem. The hereditary ataxias can be grouped into two general genetic categories, based on either autosomal recessive or autosomal dominant inheritance. Friedreich ataxia is an autosomal recessive disease, the gene for which has been assigned to chromosome 9q (Hanauer et al. 1990). The gene for one subtype of the dominantly inherited spinocerebellar ataxias (SCA1) has been localized to the short arm of chromosome 6 near the human leukocyte antigen (HLA) complex (Yakura et al. 1974; Jackson et al. 1977; Morton et al. 1980; Nino et al. 1980; Pederson et al. 1980; Whittington et al. 1980; Haines et al. 1984).

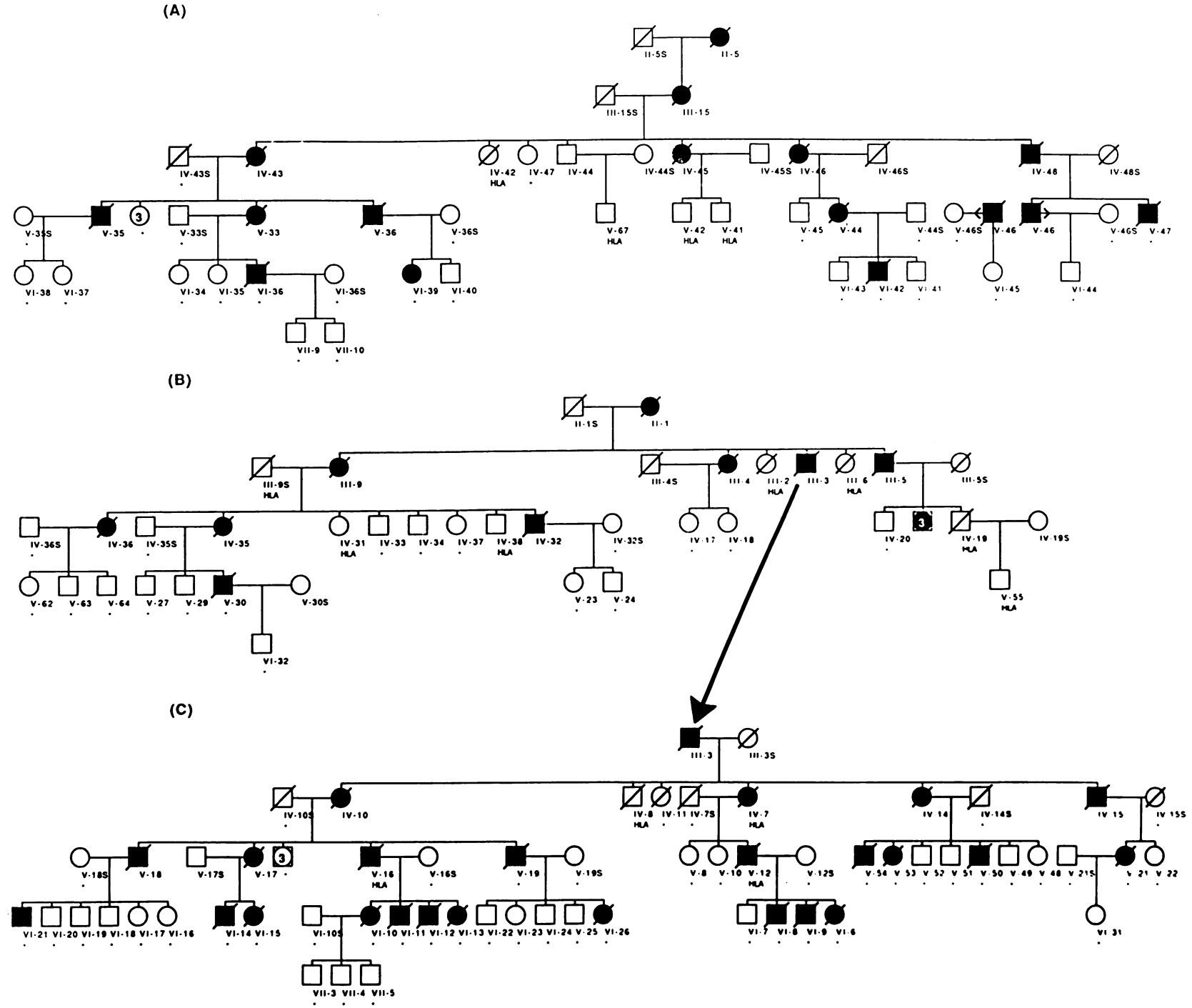
Received September 26, 1990; final revision received March 4, 1991.

Address for correspondence and reprints: Harry T. Orr, Ph.D., Department of Laboratory Medicine and Pathology, University of Minnesota, Box 206 UMHC, 420 Delaware Street, S.E., Minneapolis, MN 55455.

0002-9297/91/4901-0005$02.00

Individuals with SCA1 suffer from a combination of neurological deficits that include progressive gait ataxia, dysarthria, dysphagia, and generalized incoordination. The most consistent pathological alteration is a marked degeneration of cerebellar Purkinje neurons (Landis et al. 1974). Age at onset of symptoms is usually 20–50 years (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 reported (Zoghbi et al. 1988*b*). The disease progresses over a period of 10–30 years, ultimately resulting in death, usually due to recurrent pneumonia.

Conflicting evidence as to an HLA-distal or HLA-proximal location for SCA1 has been reported elsewhere. In one large North American kindred, we and others reported that SCA1 is distal to HLA (Haines and Trofatter 1986; Rich et al. 1987), while data from another kindred (Zoghbi et al. 1989) suggested a proximal location. To address this discrepancy, we recruited a second large SCA1 kindred originally described by Nino et al. (1980). Linkage analyses using these two large SCA1 kindreds were performed with additional markers from the region telomeric to HLA, including D6S88 (Duvick et al. 1990) and D6S89 (Litt and Luty 1990).


(A)
II-5S
II-5
III-15S
III-15
IV-43S
IV-43
IV-42
HLA
IV-47
IV-44
IV-44S
IV-45
IV-45S
IV-46
IV-46S
IV-48
IV-48S
V-33S
V-35
3
V-33S
V-33
V-36
V-36S
V-67
HLA
V-42
HLA
V-41
HLA
V-45
V-44
V-44S
V-46S
V-46
V-46
V-46S
V-47
VI-38
VI-37
VI-34
VI-35
VI-36
VI-36S
VI-39
VI-40
VI-43
VI-42
VI-41
VI-45
VI-44
VII-9
VII-10
(B)
II-1S
II-1
III-9S
HLA
III-9
III-4S
III-4
III-2
HLA
III-3
III-6
HLA
III-5
III-5S
IV-36S
IV-36
IV-35S
IV-35
IV-31
HLA
IV-33
IV-34
IV-37
IV-38
HLA
IV-32
IV-32S
IV-17
IV-18
IV-20
3
IV-19
HLA
IV-19S
V-62
V-63
V-64
V-27
V-29
V-30
V-30S
V-23
V-24
V-55
HLA
VI-32
(C)
III-3
III-3S
IV-10S
IV-10
IV-8
HLA
IV-11
IV-7S
IV-7
HLA
IV-14
IV-14S
IV-15
IV-15S
V-18S
V-18
V-17S
V-17
3
V-16
HLA
V-16S
V-19
V-19S
V-8
V-10
V-12
HLA
V-12S
V-54
V-53
V-52
V-51
V-50
V-49
V-48
V-21S
V-21
V-22
VI-21
VI-20
VI-19
VI-18
VI-17
VI-16
VI-14
VI-15
VI-10S
VI-10
VI-11
VI-12
VI-13
VI-22
VI-23
VI-24
VI-25
VI-26
VI-7
VI-8
VI-9
VI-6
VI-31
VII-3
VII-4
VII-5

Material and Methods

SCA1 Kindreds

Kindred 1 was initially designated as the Schut-Swier kindred (Gray and Oliver 1941) and kindred 2 the Sevenski kindred (Nino et al. 1980). Both kindreds have been shown to have an autosomal dominant, HLA-linked form of SCA1 (Nino et al. 1980; Haines et al. 1984). Pedigrees for the two kindreds are shown in figures 1 and 2. Table 1 presents the general clinical features of these kindreds.

Centre d'Etude du Polymorphisme Humain (CEPH) Reference Families

The panel of 40 families (CEPH) (Marx 1985) was analyzed for the linkage of the D6S88 marker with known markers from human chromosome 6p.

HLA Typing

Data for HLA-A in kindred 1, which had been determined in previous studies (Haines et al. 1984; Wilkie et al., in press), were included in the linkage analyses described below. HLA data from 63 individuals from kindred 2 who were typed by Nino et al. (1980), along with 48 newly identified members of the kindred, were also included in the linkage analyses. Additional HLA typings were performed by the HLA Typing Laboratory at the University of Minnesota, according to the method of Hopkins and MacQueen (1981).

DNA Isolation and Southern Analysis

Blood samples obtained from individuals of kindreds 1 and 2 were used to establish lymphoblastoid cell lines by Epstein-Barr virus transformation. Genomic DNA was isolated according to a method described elsewhere (Bell et al. 1981). The DNA was digested and transferred to a Zetabind nylon membrane according to a method described elsewhere (Rich et al. 1987). The D6S88 probe detects a three-allele polymorphism after digestion of genomic DNA with the restriction enzyme *Eco*RI (Duvick et al. 1990). A cDNA clone for the F13A gene (Grundmann et al. 1986) was provided by Ulrich Grundmann (Behringerke AG, Marburg, Germany). The polymorphisms detected after digestion of genomic DNA with *Bam*HI and *Bcl*I were used (Zoghbi et al. 1988*a*). DNA probes were labeled with alpha-^{32}P dCTP (3,000 Ci/mmol) by the random primer technique described by Feinberg and Vogelstein (1983). Hybridization and wash conditions were as described elsewhere (Rich et al. 1987).

PCR Amplification and Analysis of the D6S89 Marker

The D6S89 locus contains a highly polymorphic, GT-repeat sequence which is amplified by PCR using the 1717 and 1718 primers (Litt and Luty 1990). Using a subpanel of the CEPH families, Litt and Luty (1990) reported that D6S89 was distal of HLA and proximal of F13A. For D6S89, they described 13 alleles of 199–227 nucleotides. In the kindreds we examined, 18 different alleles of 177–213 nucleotides were observed. The 1717 primer was end-labeled with gamma-^{32}P ATP (3,000 Ci/mmol) before the D6S89 sequence was amplified by PCR. The PCR reaction was performed using 0.5 pmol of the 1717 primer labeled with 0.5 μCi of gamma-^{32}P ATP in addition to 10 pmol of unlabeled 1717 and 1718. The PCR conditions used have been described elsewhere (Litt and Luty 1990). PCR products were analyzed on 53 × 22-cm, 6% acrylamide (5.8% acrylamide, 0.2% N, N′-methylenebisacrylamide), denaturing, sequencing gels. M13 sequencing ladders were run with the PCR products as molecular-weight markers. After electrophoresis, gels were dried and exposed to Kodak XAR-5 film with one Dupont Lightning-Plus intensifying screen for 1–16 h.

Data Analysis

The data generated by HLA serology, as well as by RFLP and PCR analysis, were analyzed for linkage to SCA1 by using the computer package LINKAGE, version 4.7 (Lathrop et al. 1984). Age-dependent penetrances were assigned according to a method described elsewhere (Ott 1974; Haines et al. 1984). For each kindred, a set of age-dependent risk classes was constructed. Since the age-at-onset distributions for the two kindreds were of the same shape but were displaced by a mean of 12 years, corrections were made specific to each kindred.

Linkage analysis with the D6S89 marker was sim-

Figure 1 Abbreviated pedigree of kindred 1 (Schut-Swier). Blackened circles (females) and blackened squares (males) represent affected individuals. Pedigree numbers are listed below each individual. A small black dot indicates that complete data are available for that person. Individuals for which only HLA typing was done are marked "HLA."

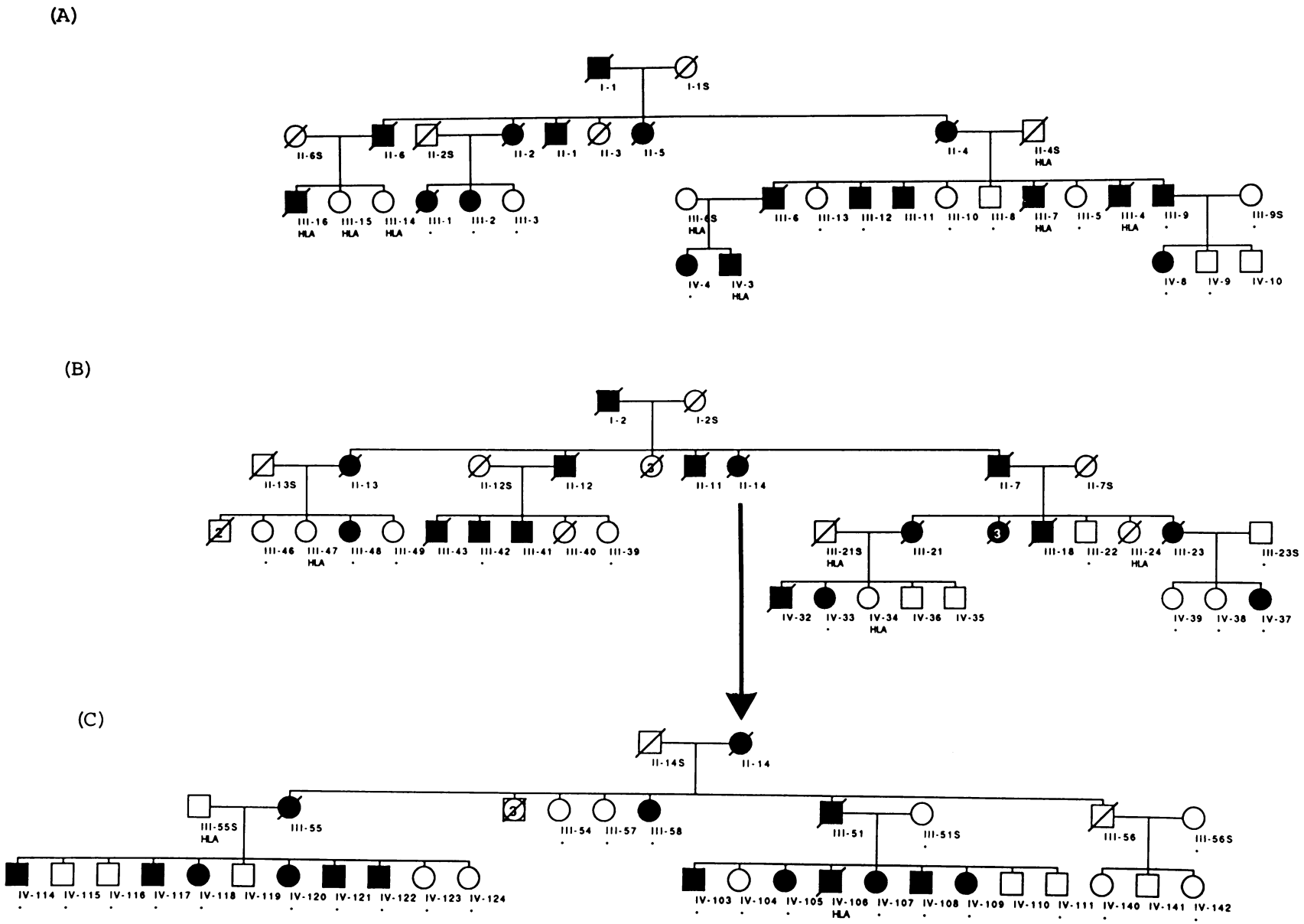


Figure 2 Abbreviated pedigree of kindred 2 (Sevenski). Blackened circles (females) and blackened squares (males) represent affected individuals. Pedigree numbers are listed below each individual. A small black dot indicates that complete data are available for that person. Individuals for which only HLA typing was done are marked "HLA."

Table 1

General Features of SCA1 Kindreds

	Kindred 1	Kindred 2
Age at onset (range)	26.3 (15–35) years	38.8 (26–59) years
Duration of disease	13.1 ± 0.7 years	25.4 ± 3.3 years
Age at death	39.3 ± 1.0 years	61.9 ± 3.1 years
No. of kindred members:		
Total	162	181
Affected (alive)	45 (14)	51 (28)
At risk	60	79
No. of cell lines:		
Affected individuals	14	19
Total	93	99

Table 2

Allelic Frequency for 6p Markers

Locus, Marker, and Allele (size)	Frequency	
	Kindred 1	Kindred 2
D6S88, 1-10-2 (*Eco*RI):		
1 (16.8 kb)	.53	.45
2 (14.8 kb)	.04	.00
3 (11.2 kb)	.43	.55
D6S89, primers 1717 and 1718 (PCR):		
1 (213 bp)	.00	.01 (E)
2 (211 bp)	.00	.02 (E)
3 (209 bp)	.00	.03 (E)
4 (207 bp)	.08 (E)	.02 (E)
5 (205 bp)	.17 (C)	.13 (D)
6 (203 bp)	.02 (E)	.07 (E)
7 (201 bp)	.00	.23 (A)
8 (199 bp)	.22 (A)	.06 (E)
9 (197 bp)	.20 (B)	.09 (B)
10 (195 bp)	.05 (E)	.03 (E)
11 (193 bp)	.06 (E)	.01
12 (191 bp)	.03 (E)	.05 (E)
13 (187 bp)	.00	.02 (E)
14 (185 bp)	.16 (D)	.20 (C)
15 (183 bp)	.00	.00
16 (181 bp)	.00	.00
17 (179 bp)	.01 (E)	.01 (E)
18 (177 bp)	.00	.02 (E)
F13A, cDNA (*Bam*HI):		
A1 (13.0 kb)	ND	.69
A2 (11.5 kb)	ND	.31
B1 (5.5 kb)	ND	.35
B2 (2.9 and 2.8 kb)	ND	.65
F13A, cDNA (*Bcl*I):		
1 (13.0 kb)	.01	.00
2 (11.5 kb)	.01	.02
3 (10.5 kb)	.08	.07
4 (9.8 kb)	.35	.15
5 (9.0 kb)	.02	.02
6 (8.8 kb)	.53	.74

Note.—ND = not determined.

plified in the two families by redefining the 18-allele system to a five-allele system. The alleles that segregated with the disease in each family were redefined as the A alleles. The three other most frequent alleles in each kindred were designated B–D. The other, less frequent alleles were grouped together and defined as the E allele (see table 2).

Pairwise linkage analyses were performed with the MLINK and ILINK programs. Multipoint analyses were performed with the LINKMAP program. For each fixed order, $\log_{10}$ likelihoods were calculated when SCA1 was inserted into each interval, and the most likely position was determined. For each fixed order, the $\log_{10}$ likelihood of SCA1 being unlinked was also calculated. The $\log_{10}$ likelihood of SCA1 being unlinked was subtracted from the $\log_{10}$ likelihood of SCA1 being in the interval most likely to provide a multipoint lod score (Z_{max}).

For pairwise linkage results, support-interval limits for each recombination fraction (θ) were obtained using the ($Z_{max} - 1$) method (Conneally et al. 1985). Genetic distances were calculated by using the method of Kosambi (1944). The test for a sex difference in the θ values was performed by using a χ^2, statistic, with $\chi^2 = 2 (\ln 10) [Z(\theta_m, \theta_f) - Z (\theta = \theta_m = \theta_f)]$, where $Z(\theta_m, \theta_f)$ is the overall Z_{max} for arbitrary θ_m and θ_f, while $Z (\theta = \theta_m = \theta_f)$ is the Z_{max} constrained to $\theta_m = \theta_f$. Under homogeneity (H1), χ^2 approximates a χ^2 with 1 df. Rejection of homogeneity occurs when $\chi^2 > 3.84$.

Heterogeneity analysis was carried out using the admixture test of Smith (1963), as implemented in the HOMOG program of Ott (1983). Two null hypotheses of homogeneity can be considered. In the first, the data are used to evaluate the alternative "heterogeneity" hypothesis H2—i.e., that linkage is present in only

Table 3

Pedigree Size and Number of Individuals Analyzed

Locus/Marker	Kindred 1 (n = 162)	Kindred 2 (n = 181)
HLA-A	138	111[a]
D6S88	93	97
D6S89	93	99
F13A/IEF[b]	93	ND
F13A/cDNA, *BclI*	93	99
F13A/cDNA, *Bam*HI	ND	95

NOTE.—ND = not determined.
[a] Data include typing of 63 individuals by Nino et al. (1980).
[b] Data from Wilkie et al. (in press).

a proportion of cases (with proportionality constant α, where $\alpha < 1$ and $\theta < 1/2$)—versus the null "homogeneity" hypothesis (H0) of no linkage. The second analysis considers the secondary null "homogeneity" hypothesis H1—i.e., that linkage is present ($\theta < 1/2$) and that all cases reflect this homogeneous process ($\alpha = 1$)—versus the alternative "heterogeneity" hypothesis (H2).

Results

Polymorphisms Detected and Allele Frequencies

For each SCA1 kindred, the numbers of individuals analyzed for particular markers are summarized in table 3. Allele frequencies for the D6S88, D6S89, and F13A markers are listed in table 2. To increase informativeness at the F13A locus, haplotypes were formed in kindred 1 by using the *BclI* polymorphisms and the isoelectric protein polymorphisms obtained by Wilkie et al. (in press). In kindred 2, F13A haplotypes were constructed by using the *BclI* and *Bam*HI polymorphisms.

Genetic Linkage Analysis

SCA linkage heterogeneity.—The hypothesis that the SCA1 gene in kindred 1 is in the same location as that of kindred 2 was tested using the HOMOG procedure of Ott (1983). The hypothesis H2 (linkage in a proportion of cases, α) was strongly favored over the null hypothesis (H0) of no linkage for all markers ($\chi^2_2 = 34.63$, $P < .001$). For each marker, the proportionality parameter, α, converged to unity in the H2 hypothesis. As expected from these results, the H1 hypothesis (linkage and homogeneity) could not be rejected over the H2 hypothesis for each marker ($\chi^2_1 = 0$, $P = .500$). These data strongly support the conclusions of linkage and linkage homogeneity and that a mutation in the same locus is likely responsible for the disease in both kindreds. Therefore, for the two SCA1 kindreds, the results of the two- and three-point linkage analyses between the 6p markers have been combined throughout.

Pairwise linkage data.—Pairwise Z scores were calculated, using the two SCA1 kindreds and several θ values, for the markers SCA1 and HLA-A, SCA1 and D6S88, SCA1 and D6S89, and SCA1 and F13A (table 4). Significant evidence for linkage with SCA1 was found with all markers tested. Z_{max} scores were found between SCA1 and HLA-A at $\theta = .198$ ($Z = 12.36$), between SCA1 and D6S88 at $\theta = .001$ ($Z = 10.42$), between SCA1 and D6S89 at $\theta = .014$ ($Z = 20.80$), and between SCA1 and F13A at $\theta = .190$ ($Z = 8.44$). In these two kindreds, one recombinant (individual III-42 of kindred 2) was inferred between SCA1 and D6S89. This individual carries the same HLA-A allele as do both his affected father and affected grandfather. No information about which alleles for D6S88 and D6S89 are present in his father and grandfather are available. The suspected recombinant individual does, however, carry allele 1 at the D6S88 locus (present in all affected members of this kindred) but does not carry

Table 4

Pairwise Linkage for 6p Loci in SCA1 Kindreds

Marker Pair	θ .00	.05	.10	.20	.30	.40	θ_{max}	Z_{max}	Support Interval[a]
SCA1 and HLA-A	$-\infty$	3.87	9.55	12.12	10.38	6.18	.198	12.36	.13–.27
SCA1 and D6S88	10.42	9.66	8.72	6.53	4.12	1.66	.001	10.42	.00–.07
SCA1 and D6S89	17.33	20.11	18.94	14.68	9.90	4.44	.014	20.80	.00–.06
SCA1 and F13A	$-\infty$	2.83	6.77	8.42	7.00	3.90	.190	8.44	.12–.28

[a] Z_{max} − 1 support interval for θ.

Table 5

Two-Point Linkage Data for Chromosome 6p Markers—CEPH Families

Marker Pair	$\theta_m = \theta_f$	Z_{max}	θ_m	θ_f	Z_{max}	χ^2
HLA-A and D6S88	.125	26.46	.104	.162	26.66	.92
HLA-A and D6S89[a]	.155	42.96	.089	.222	46.26	15.20[b]
HLA-A and F13A	.291	8.80	.255	.325	9.14	1.57
D6S88 and D6S89	.084	37.03	.075	.095	37.13	.46
D6S88 and F13A	.176	10.43	.159	.206	10.51	.37
D6S89 and F13A[a]	.151	30.67	.141	.160	30.73	.28

[a] Based on the data generated by Zoghbi et al. (1991).

[b] Rejection of assumption of homogeneity ($\theta_m = \theta_f$); that is, the $\chi^2 = 15.20$ should occur by chance, $P < .001$.

allele A for D6S89 (present in all other affected individuals). These data are, therefore, consistent with a recombination having occurred between SCA1 and D6S89, a recombination which did not affect HLA-A or D6S88. No obligate recombinants between D6S88 and SCA1 were observed.

6p Markers on the CEPH Map.—To facilitate ordering SCA1 relative to the 6p markers in our kindreds and to more firmly place D6S88 on the 6p map, D6S88 was mapped using the CEPH families. Two- and three-point linkage analyses were performed between D6S88 and several other markers already on the CEPH map: HLA-A (provided by Dr. Howard Cann of CEPH), F13A (Zoghbi et al. 1988*a*), and D6S89 (Litt and Luty 1990; Zoghbi et al. 1991). For the CEPH data, the Z_{max} scores and θ values for the two-point analysis between D6S88 and HLA-A, between D6S88 and D6S89, between D6S88 and F13A, between D6S89 and HLA-A, between D6S89 and F13A, and between HLA-A and F13A are summarized in table 5. The results for the three-point analyses between HLA, D6S88, and F13A; between HLA-A, D6S89, and F13A; between HLA-A, D6S88, and D6S89; and between D6S88, D6S89, and F13A are shown in table 6. The order HLA-A–D6S88–F13A is favored over the next most likely order (D6S88–HLA-A–F13A) by odds of 10^3:1. The order HLA-A–D6S89–F13A is favored over the next most likely order (D6S89–HLA-A–F13A) by odds of 10^{19}:1. The order HLA-A–D6S88–D6S89 is favored over the next most likely order (D6S88–D6S89–HLA-A) by odds of

Table 6

Three-Point Linkage Data: CEPH Families

Order (θ)	Z_{max}[a]	Relative Odds	Odds in Favor
HLA-A (.137) D6S88 (.210) F13A	40.99	7.6×10^{12}	3.3×10^3:1
D6S88 (.120) HLA-A (.274) F13A	37.48	2.3×10^9	
D6S88 (.129) F13A (.242) HLA-A	28.11	1	
HLA-A (.159) D6S89[b] (.154) F13A	75.45	3.5×10^{22}	3.1×10^{19}:1
D6S89[b] (.151) HLA-A (.263) F13A	56.23	2.1×10^3	
D6S89[b] (.127) F13A (.232) HLA-A	52.90	1	
HLA-A (.120) D6S88 (.083) D6S89	90.02	1.7×10^{12}	1.3×10^5:1
D6S88 (.085) D6S89 (.158) HLA-A	84.89	1.3×10^7	
D6S88 (.111) HLA-A (.148) D6S89	77.78	1	
D6S88 (.083) D6S89 (.150) F13A	70.45	3.4×10^{13}	5.0×10^4:1
D6S89 (.080) D6S88 (.146) F13A	65.49	3.7×10^8	
D6S89 (.122) F13A (.111) D6S88	56.92	1	

[a] Multipoint (Ott's generalized) lod score.

[b] Based on data generated by Zoghbi et al. (1991).

Table 7

Three-Point Linkage Analysis of SCA1 Kindreds 1 and 2

Order (θ)	Z_{max}[a]	Relative Odds	Odds in Favor
SCA1 (.197) HLA-A (.250) F13A	22.19	10.2	
HLA-A (.142) SCA1 (.108) F13A	28.51	2.1×10^7	2.1×10^6:1
HLA-A (.250) F13A (.179) SCA1	21.18	1	
SCA1 (.197) HLA-A (.155) D6S89.....	27.40	1	
HLA-A (.140) SCA1 (.015) D6S89.....	43.24	6.9×10^{15}	1.0×10^3:1
HLA-A (.155) D6S89 (.018) SCA1.....	40.23	6.8×10^{12}	

[a] Multipoint (Ott's generalized) lod score.

> 10^5:1, and the order D6S88–D6S89–F13A is favored over the next most likely order (D6S89–D6S88–F13A) by odds of >10^4:1. For the CEPH families, both the two- and three-point linkage data overwhelmingly support the marker order 6pcen–HLA-A–D6S88–D6S89–F13A–6pter. The hypothesis of homogeneity of θ values ($\theta_m = \theta_f$) was rejected for only one pair of markers—HLA-A and D6S89 (θ_m = .089, θ_f = .222; $\theta_m = \theta_f$ = .155). All other analyses of marker pairs did not reject the homogeneity ($\theta = \theta_m = \theta_f$) hypothesis (table 5).

Three-point linkage analysis.—The distances between HLA-A, D6S88, D6S89, and F13A were fixed for three-point analysis in the two SCA1 kindreds, on the basis of the two-point $\theta_m = \theta_f$ values in the CEPH families. Results of the three-point linkage analysis between HLA-A, SCA1, and F13A are given in table 7 and figure 3. The HLA-A–SCA1–F13A order is favored over the next most likely order (SCA1–HLA-A–F13A) by odds of 10^6:1. These data confirm earlier reports, by Haines and Trofatter (1986) and Rich et al. (1987), of an HLA-A-distal position for the SCA1 locus. The results of the three-point linkage analysis between HLA-A, SCA1, and D6S89 are shown in figure 4 and table 7. The HLA-A–SCA1–D6S89 order is favored over the next most likely order (HLA-A–D6S89–SCA1) by odds of 1,000:1. The three-point analyses both for the markers D6S88, SCA1, and D6S89 and for the markers SCA1, D6S89, and F13A were uninformative for determining the

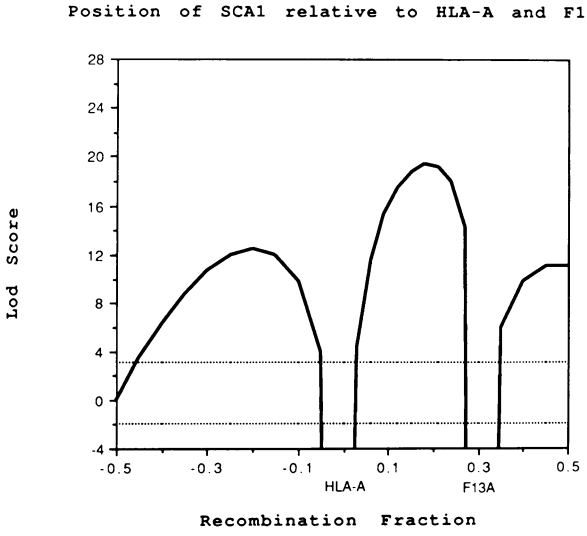


Figure 3 Three-point linkage analysis of SCA1, HLA-A and F13A, for kindreds 1 and 2. The solid line traces the lod score at various θ values for the SCA1 locus relative to HLA-A and F13A.

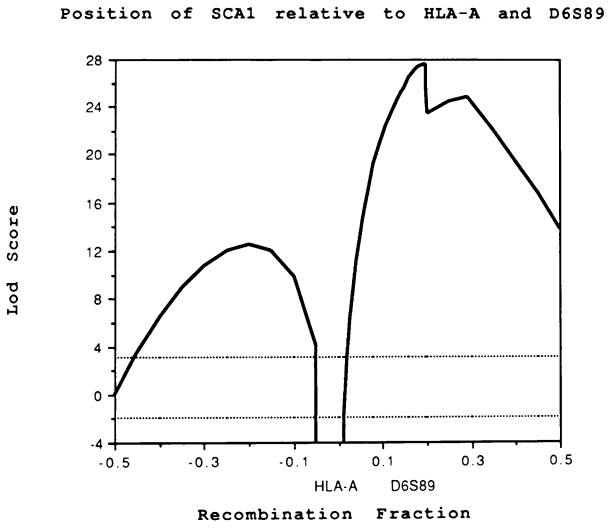


Figure 4 Three-point linkage analysis of SCA1, HLA-A, and D6S89, for kindreds 1 and 2. The solid line traces the lod score at various θ values for the SCA1 locus relative to HLA-A and D6S89.

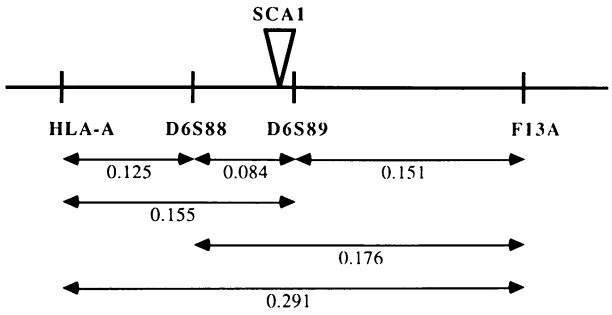


Figure 5 Map of SCA1 region of 6p between HLA-A and F13A. Pairwise distances between HLA-A, D6S88, D6S89, and F13A are based on θ values observed in the CEPH families. SCA1 was positioned on the CEPH map on the basis of the θ value at the Z_{max}, from kindreds 1 and 2, between SCA1 and D6S89.

position of SCA1. Four- and five-point analyses were not performed, because of the large amount of computer time required.

A map summarizing the relative positions of the markers HLA-A, D6S88, D6S89, and F13A is shown in figure 5. This map is based on the two- and three-point linkage analyses in the CEPH families, as well as on the positioning of the SCA1 gene, a positioning that, in turn, is based on the three-point linkage analyses with the HLA-A, SCA1, and D6S89 markers.

Discussion

Results of the three-point analysis between the markers HLA-A, SCA1, and F13A overwhelmingly favor (by odds of 6×10^6:1 over the next most likely order) the conclusion that SCA1 is distal of HLA-A and proximal of F13A. These results corroborate previous studies (using kindred 1) by Haines and Trofatter (1986) and Rich et al. (1987). In addition, the ambiguity between our HLA-distal location and the HLA-proximal location for SCA1 in the kindred described by Zoghbi et al. (1989) has been resolved, in large part because of the finding of close linkage to the highly informative D6S89 marker (Zoghbi et al. 1991).

SCA1 has been further localized by the results from the three-point linkage analyses between HLA-A, SCA1, and D6S89. The HLA-A–SCA1–D6S89 order is favored by odds of 1,000:1 over the next most likely order. The localization of SCA1 relative to D6S89 is based on a single recombinant. To insure that this is not the result of a laboratory error, the individual was resampled and retyped; identical results were obtained. These results further define the SCA1 location as being centromeric and genetically very close to the highly informative D6S89 marker. The θ value between D6S89 and SCA1 is .014 at Z_{max} = 20.80. For D6S89, Litt and Luty (1990) reported a 92% heterozygosity and a PIC of .88. In the two kindreds under study in the present report, 18 different alleles for D6S89 were observed. Results of the three-point analysis support a distal location for D6S89, relative to SCA1.

Although no recombinants between D6S88 and SCA1 were observed in the two SCA1 kindreds (θ = .001, Z_{max} = 10.42), the D6S88 marker is not as informative as D6S89. Because of the limits inherent in establishing a genetic map by using the two SCA1 kindreds, the θ = .08 value between D6S88 and D6S89 (tables 5 and 6) found in the CEPH panel was used to establish the recombination map shown in figure 5. In the two kindreds, the position of SCA1 relative to D6S89 is far more reliable (because of the high degree of informativeness of D6S89) than is the distance between SCA1 and D6S88. Therefore, SCA1 was placed on the CEPH map, relative to D6S88 and D6S89, on the basis both of the three-point linkage analyses between HLA-A, SCA1, and D6S89 and of the two-point analysis between SCA1 and D6S89. On the CEPH map, D6S88 and D6S89 are separated by 8 cM, and the marker order 6pcen–HLA-A–D6S88–D6S89–F13A–6pter has been firmly established by a series of three-point analyses (table 6). Because our most reliable linkage data for SCA1 in kindreds 1 and 2 position SCA1 approximately 1.5 cM centromeric of D6S89, we conclude that D6S88 must be centromeric of SCA1. Therefore, our data support the conclusion that SCA1 lies within the 8-cM region flanked by the D6S88 and D6S89 markers.

A number of non-HLA linked, autosomal dominant SCA kindreds have been reported in the literature (Kumar et al. 1986; Auburger et al. 1990). Because D6S89 is tightly linked to the chromosome 6p form of SCA and is a highly informative marker, newly identified kindreds can be quickly screened with D6S89 to determine whether a particular kindred reflects the same autosomal dominant SCA as exemplified by kindreds 1 and 2.

The identification of the markers D6S88 and D6S89, which flank SCA1, will greatly facilitate efforts to isolate additional DNA markers from the SCA1 subregion of chromosome 6p. It is anticipated that before rapid progress can be made by using physical mapping techniques, such as PFGE and YAC cloning, a marker must be identified that, compared with

D6S89, maps equally close to—but on the other side of—the SCA1 locus. Currently, strategies to identify additional DNA markers for the SCA1 region are being pursued.

Acknowledgments

We are grateful to members of the Schut-Swier and Sevenski kindreds for their participation in this study. We thank Dr. Grundmann for providing us with the F13A cDNA clone, and we thank Dr. James Weber for help in running the CEPH data analysis program. We are most grateful to Dr. Huda Zoghbi and her collaborators for communicating their results prior to publication. This work was supported by NIH grants NS22920 (to H.T.O.) and RR01632 (to S.S.R.) and by grants from the Muscular Dystrophy Association (to H.T.O. and L.P.W.R.) and from the National Ataxia Foundation (to H.T.O.).

References

Auburger G, Diaz GO, Capote RF, Sanchez SG, Perez MP, Estrada del Cueto M, Meneses MG, et al (1990) Autosomal dominant ataxia: genetic evidence for locus heterogeneity from a Cuban founder-effect population. Am J Hum Genet 46:1163–1177

Bell GI, Kavam J, Rutter W (1981) Polymorphic DNA region adjacent to the 5′ end of the human insulin gene. Proc Natl Acad Sci USA 78:5759–5763

Conneally PM, Edwards JH, Kidd KK, Lalouel JM, Morton NE, Ott J, White R (1985) Report of the Committee of Methods of Linkage Analysis and Reporting. Cytogenet Cell Genet 40:356–359

Currier RD, Glover G, Jackson JF, Tipton AC (1972) Spinocerebellar ataxia: study of a large kindred. Neurology 22:1040–1043

Duvick L, Rich SS, Orr HT (1990) A polymorphic DNA probe, p1–10–2, from chromosome 6. Nucleic Acids Res 18:3105

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267

Gray RC, Oliver CP (1941) Marie's hereditary cerebellar ataxia (olivopontocerebellar atrophy). Minn Med 24: 327–335

Grundmann U, Amann E, Zettlemeisel G, Kuppa HA (1986) Characterization of cDNA coding for human factor XIIIa. Proc Natl Acad Sci USA 83:8024–8028

Haines JL, Schut LJ, Weitkamp LR, Thayer M, Anderson VE (1984) Spinocerebellar ataxia in large kindred: age at onset, reproduction, and genetic linkage studies. Neurology 34:1542–1548

Haines JL, Trofatter JA (1986) Multipoint linkage analysis of spinocerebellar ataxia and markers on chromosome 6. Genet Epidemiol 3:399–405

Hanauer A, Chery M, Fujita R, Driesel AJ, Gilgenkrantz S, Mandel JL (1990) The Friedreich ataxia gene is assigned to chromosome 9q13-q21 by mapping of tightly linked markers and shows linkage disequilibrium with D9S15. Am J Hum Genet 46:133–137

Hopkins KA, MacQueen JM (1981) Basic microlymphocytotoxicity technique. In: Zachary AA, Braun WE (eds) The AACHT laboratory manual. American Association for Clinical Histocompatibility Testing, New York, pp 11-1-1-11-2-1

Jackson JF, Currier RD, Terasaki PI, Morton NE (1977) Spinocerebellar ataxia and HLA linkage: risk prediction by HLA typing. N Engl J Med 296:1138–1141

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugenics 12:172–175

Kumar D, Blank CE, Gelsthorpe K (1986) Hereditary cerebellar ataxia and genetic linkage with HLA. Hum Genet 72:327–332

Landis DMD, Rosenberg RN, Landis SC, Schut LJ, Nylan WL (1974) Olivopontocerebellar degeneration: clinical and ultrastructural abnormalities. Arch Neurol 31:295–307

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Litt M, Luty JA (1990) Dinucleotide repeat polymorphism at the D6S89 locus. Nucleic Acids Res 18:4301

Marx JL (1985) Putting the human genome on the map. Science 229:150–151

Morton NE, Lalouel JM, Jackson JF, Currier RD, Yee S (1980) Linkage studies in spinocerebellar ataxia (SCA). Am J Med Genet 6:251–257

Nino HE, Noreen HJ, Dubey DP, Resch JA, Namboodiri K, Elstrom RC, Yunis EJ (1980) A family with hereditary ataxia: HLA typing. Neurology 30:12–20

Ott J (1974) Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. Am J Hum Genet 26:588–594

——— (1983) Linkage analysis and family classification under heterogeneity. Ann Hum Genet 47:311–320

Pederson L, Platz P, Ryder LP, Lamm LU, Dissing J (1980) A linkage study of hereditary ataxias and related disorders: evidence of heterogeneity of dominant cerebellar ataxia. Hum Genet 54:371–383

Rich SS, Wilkie P, Schut L, Vance G, Orr HT (1987) Spinocerebellar ataxia: localization of an autosomal dominant locus between two markers on human chromosome 6. Am J Hum Genet 41:524–531

Smith CAB (1963) Testing for heterogeneity of recombination values in human genetics. Ann Hum Genet 27:175–182

Whittington JE, Keats BJB, Jackson JF, Currier RD, Terasaki PI (1980) Linkage studies on glyoxalase I (GLO), pepsinogen (PG), spinocerebellar ataxia (SCA1), and HLA. Cytogenet Cell Genet 28:145–150

Wilkie PJ, Schut LJ, Rich SS. Spinocerebellar ataxia:

multipoint linkage analysis of genes associated with the disease locus. Hum Genet (in press)

Yakura H, Wakisaka A, Fujimotos S, Itakura K (1974) Hereditary ataxia and HLA genotypes. N Engl J Med 291: 154–155

Zoghbi HY, Daiger SP, McCall A, O'Brien WE, Beaudet AL (1988*a*) Extensive DNA polymorphism at the factor XIIIa (F13A) locus and linkage to HLA. Am J Hum Genet 42: 877–883

Zoghbi HY, Jodice C, Sandkuyl LA, Kwiatkowski TJ Jr, McCall AE, Huntoon SA, Lulli P, et al (1991) The gene for autosomal dominant spinocerebellar ataxia (SCA1) maps telomeric to the HLA complex and is closely linked to the D6S89 locus in three kindreds. Am J Hum Genet 49:000–000

Zoghbi HY, Pollack MS, Lyons LA, Ferrell RE, Daiger SP, Beaudet AL (1988*b*) Spinocerebellar ataxia: variable age of onset and linkage to HLA in a large kindred. Ann Neurol 23:580–584

Zoghbi HY, Sandkuyl LA, Ott J, Daiger SP, Pollack M, O'Brien WE, Beaudet AL (1989) Assignment of autosomal dominant spinocerebellar ataxia (SCA1) centromeric to the HLA region on the short arm of chromosome 6, using multilocus linkage analysis. Am J Hum Genet 44: 255–263