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Autosomal Dominant Ataxia: Genetic Evidence for Locus Heterogeneity from a Cuban Founder-Effect Population

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Summary

The locus for autosomal dominant ataxia with a diagnosis of olivo-ponto-cerebellar atrophy at autopsy has been previously assigned to chromosome 6p. However, evidence for two alternative locations has been reported. We have recently described a large potential founder-effect population of such patients in the Holguin province of Cuba. With an estimated 1,000 patients available for analysis, this extensive cluster of families provides a unique opportunity for the definitive localization of the genetic mutation. Linkage analysis between the disease locus in this population and markers within and flanking the HLA region on chromosome 6 were undertaken in 12 families comprising over 100 affected individuals. Despite similarity in the clinical phenotype between those families where the disease locus has been reported to be linked to the HLA locus and the Cuban patients, no evidence of linkage to this region could be demonstrated in the latter. The disease locus was excluded from a 96-cM genetic interval of the short arm of chromosome 6, encompassing the F13A1–HLA–GLO1–MUT/D6S4 loci. These data strongly support the existence of genetic heterogeneity for the disease.

Introduction

The dominantly inherited autosomal ataxias represent a clinically heterogeneous group of neurodegenerative disorders characterized by a predominantly cerebellar syndrome of progressive ataxia, dysarthria, dysmetria, and dysdiadochokinesia. Considerable variation is observed in the age at onset, rate of progression, severity, and occurrence of accessory symptoms (Brown 1892; Konigsmark and Weiner 1970; Wadia and Swami 1971; Pedersen et al. 1980; Harding 1982), although a diagnosis of olivo-ponto-cerebellar atrophy (OCPA) is generally made at autopsy. Subclassification schemes based on neuropathological (Konigsmark and Weiner 1970) and clinical criteria (Harding 1982) have therefore failed to find universal acceptance. Whether the clinical variability reflects inter- or intragenic heterogeneity will only be resolved following the chromosomal assignment and eventual identification, by molecular analysis, of the mutation(s) underlying these disorders.

The demonstration of genetic linkage to the HLA complex in families with autosomal dominant ataxia led to the initial assignment of the disease locus to chromosome 6p (Yakura et al. 1974; Jackson et al. 1977). This HLA-linked form has been named spinocerebellar ataxia 1 (SCA1). Further analysis using cloned DNA markers flanking the complex in several pedigrees of diverse origin have generated alternative evidence as to the precise location of the disease locus. In two large North American pedigrees, evidence of a distal location with respect to the HLA locus has been reported (Wilkie et al. 1987; Orr and Rich 1989), while in the pedigrees studied by Zoghbi et al. (1989) a proximal location has been suggested. Additional pedigrees have

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been described which show no evidence of close linkage to HLA. However, in view of the comparatively small pedigrees studied and the limited informativity of flanking markers used, the exclusion extended over only 6 cM (Wastiaux et al. 1978), 16 cM (Koeppen et al. 1981), 15 cM (Werdelin et al. 1984) and 14 cM (Kumar et al. 1986). Since the SCA1 locus is reported to be at approximately 20 cM distance from HLA (Rich and Orr 1989), none of these reports can provide proof of heterogeneity. In fact, Nino et al. (1980) reported exclusion of their pedigree from close linkage to HLA (7 cM), while this pedigree has now been shown to be HLA linked at 20 cM (Rich and Orr 1989). Even Sasaki et al.'s (1988) study of a number of families, a study that includes 28 cM around HLA, does not rule out the existence of a common gene locus for dominant ataxia at some 30 cM distance from HLA, as postulated by Kumar et al. (1986).

We have previously described clinical observations made on 263 patients having dominant ataxia and living in the Holguin province of Cuba. A diagnosis of OPCA has been confirmed in 11 cases coming to autopsy (Orozco et al. 1989). This finding distinguishes this population from the group of Azores patients having dominant ataxia, a group who are also of Spanish origin but in whom the inferior olive is reported to be spared (Sachdev et al. 1982). In addition to the classical cerebellar symptoms, tremor, cramps, hypotonia, abnormal reflexes, and slow/limited eye movements were commonly observed. The extremely high prevalence of the disease in this region (133/100,000 inhabitants compared with 5/100,000 in Europe) led to extensive investigation of family histories and to epidemiological studies. The families are apparently of Spanish descent, but, because of lack of documents, neither their exact origin nor the links between them could be established. All of them have come from the same geographical area in Holguin province. Dominant ataxia families are found nowhere else in Cuba. Although considerable variation in clinical presentation is seen overall, including variation in several features previously attributed as evidence of distinct clinical entities, no interfamilial differences are observed. Age at onset varied from 2 to 65 years, with 40% of patients presenting before 25 years of age. Optic atrophy/spasticity, retinopathy, dementia, and, in particular, rigidity were not part of the disease phenotype in these families (Orozco et al., in press-*a*), in spite of a significant alteration of dopamine metabolites (Orozco et al. 1989). This lack of extrapyramidal and pyramidal signs also distinguishes this population from the Machado-Joseph patients on the Azores, where these features are common presenting complaints (Rosenberg and Grossman 1989). The clinical hemogeneity of the Cuban families, together with the common geographical origin, is suggestive of a founder effect.

The quality of the pedigrees, with an estimated 1,000 living affected individuals available for analysis, and with the high probability of genetic homogeneity through a common ancestral origin for this population, offers a unique opportunity to identify the mutation responsible for this disease. Initial studies have focused on the investigation of linkage to markers flanking the HLA locus on chromosome 6.

Material and Methods

Twelve pedigrees including 111 living affected members were selected for linkage analysis (figs. 1–12). Detailed neurological examination had been previously carried out by the same author (G.O.D.), and details of the disease history had been established, extending back over 5–6 generations (Orozco et al., in press-*a*).

Conventional serotyping for the HLA-A and -B loci was performed in two families by standard microlymphocytotoxicity assay (Mittal et al. 1968); for the other pedigrees, analysis within 8 h of collection proved impossible because of their geographical isolation. Consequently, genetic linkage analysis was carried out between the disease locus and the cloned DNA markers corresponding to the loci for HLA-DQA1, F13A1 (coagulation factor XIII, A1 polypeptide), and the anonymous marker D6S4; these markers have been recommended as arbitrary reference points spanning chromosome 6p (Spence et al. 1989). Analyses with the GLO1 (glyoxylase 1) and MUT (methylmalonyl coenzyme A mutase) loci were subsequently included to increase informativity in the centromeric region, MUT and D6S4 being tightly linked (lod score = 22.64; recombination fraction = .01) (Zoghbi et al. 1988*b*). The polymorphic characteristics of the RFLPs used are shown in table 1. Because of the limited genetic pool resulting from the founder effect, allele frequencies could not be expected to reflect published values and were therefore established from those individuals who had married into the ataxia families. Allele frequencies for the GLO1 locus were set at .36 and .64. Physical assignments of the marker loci are shown in figure 13.

Venous blood samples from the family members indicated were collected into EDTA, and genomic DNA was prepared by standard techniques. Five micrograms of DNA were digested with the appropriate restriction

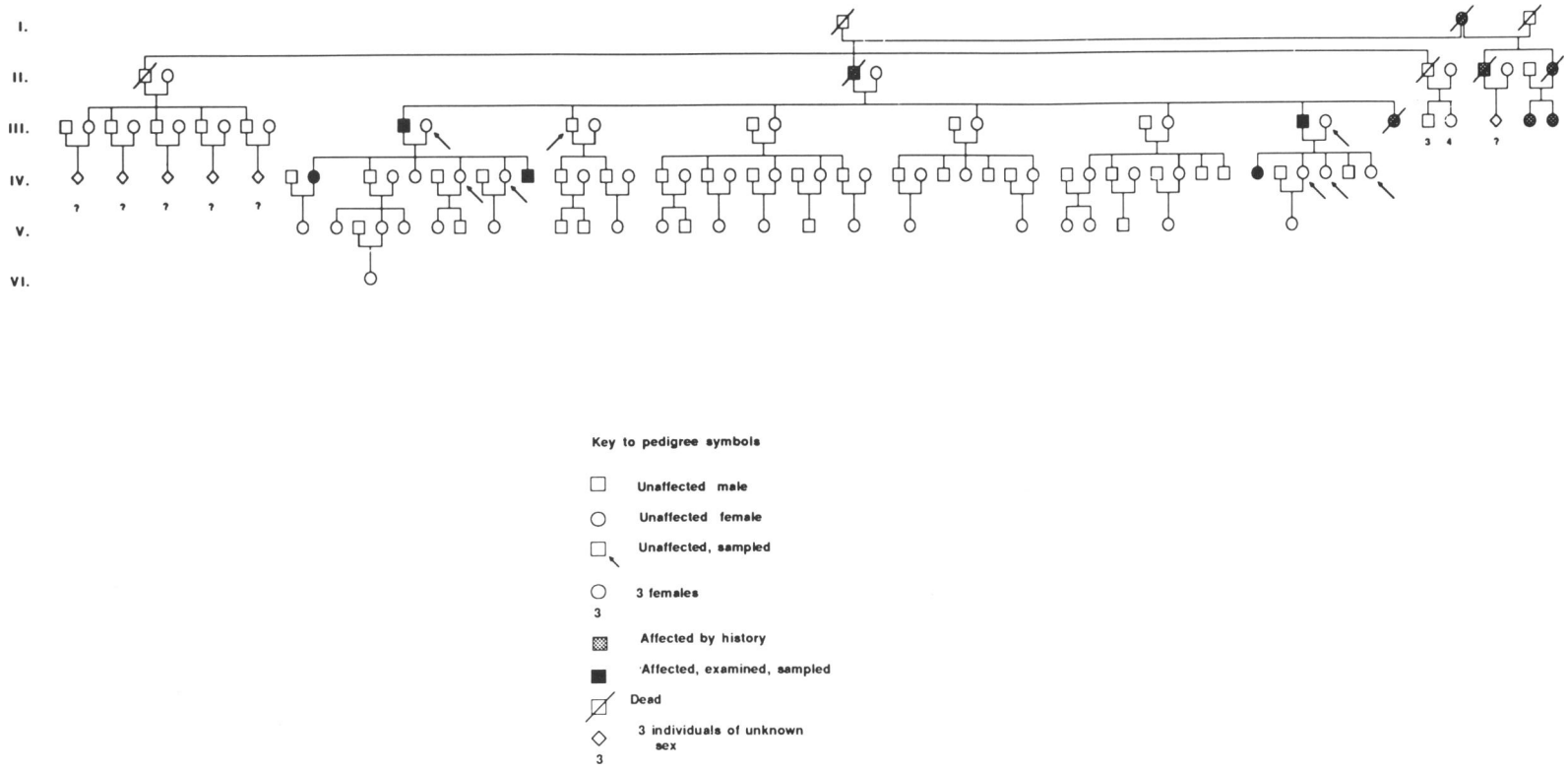


Figure 1 Pedigree LB

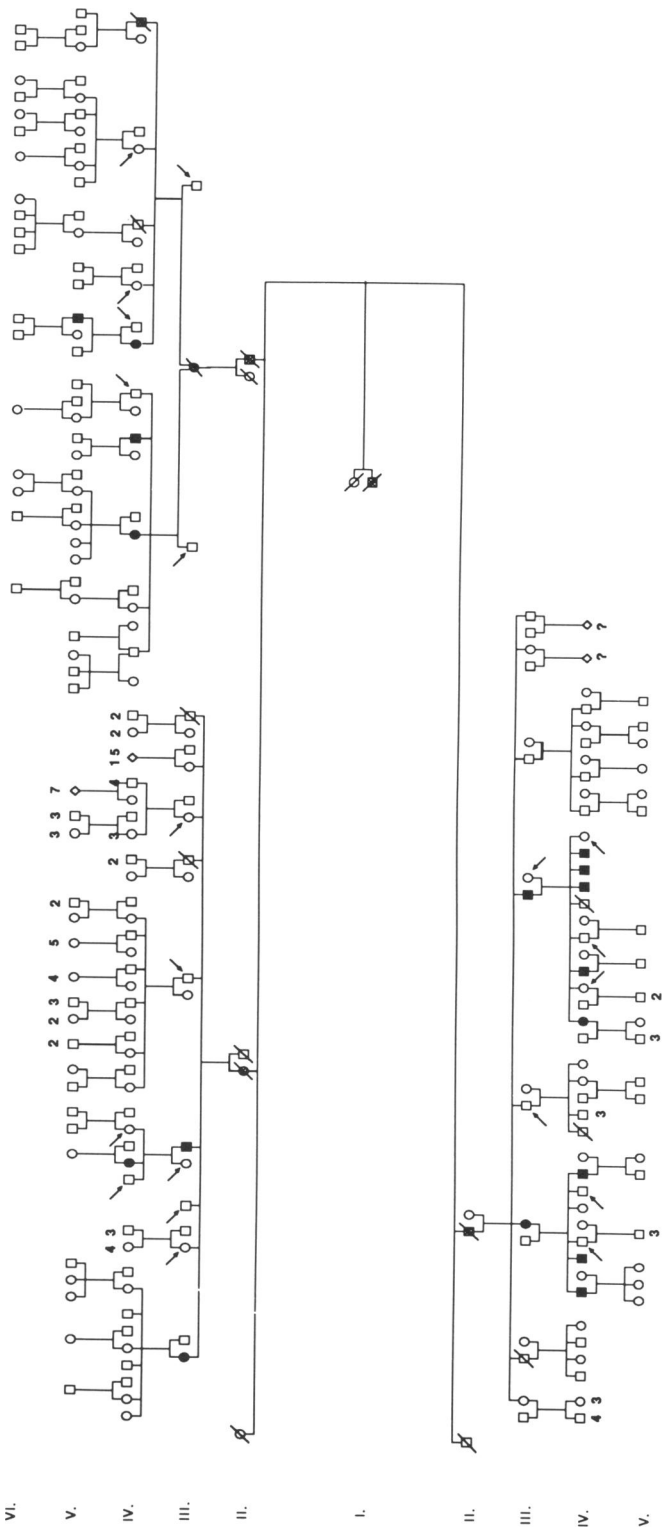


Figure 2 Pedigree PL

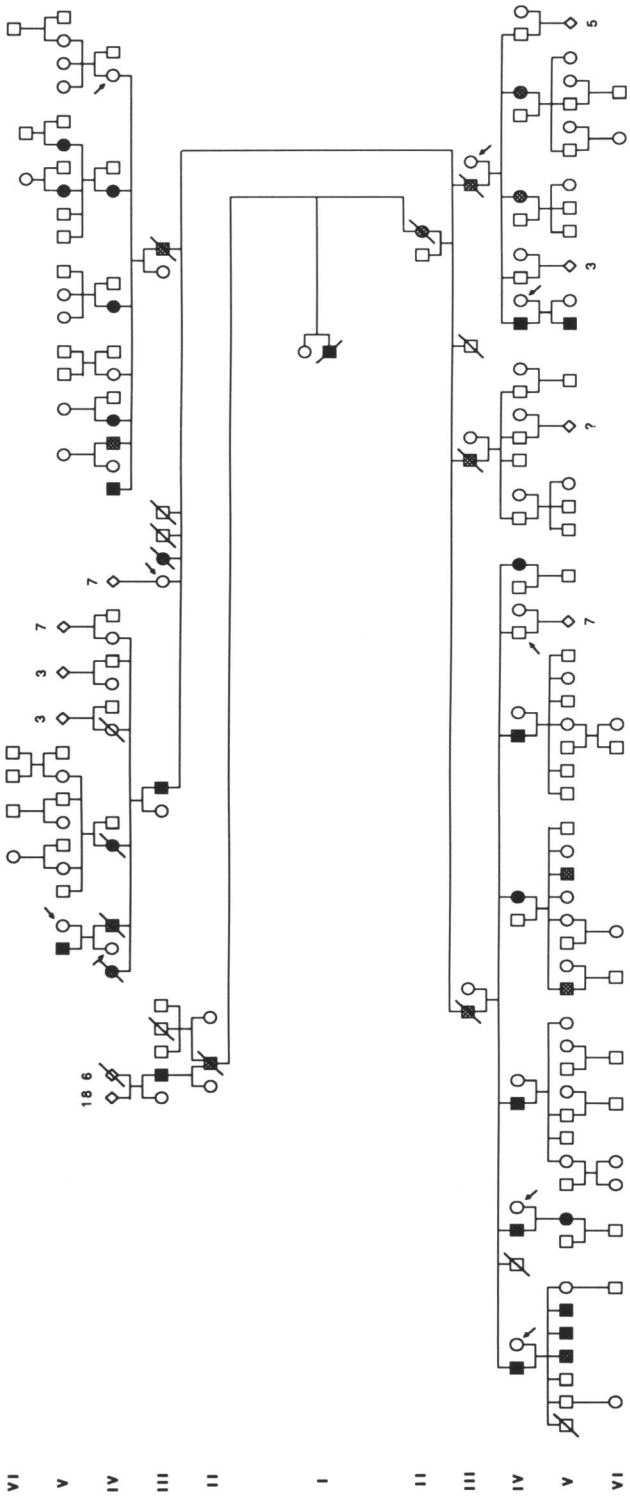


Figure 3 Pedigree Rs

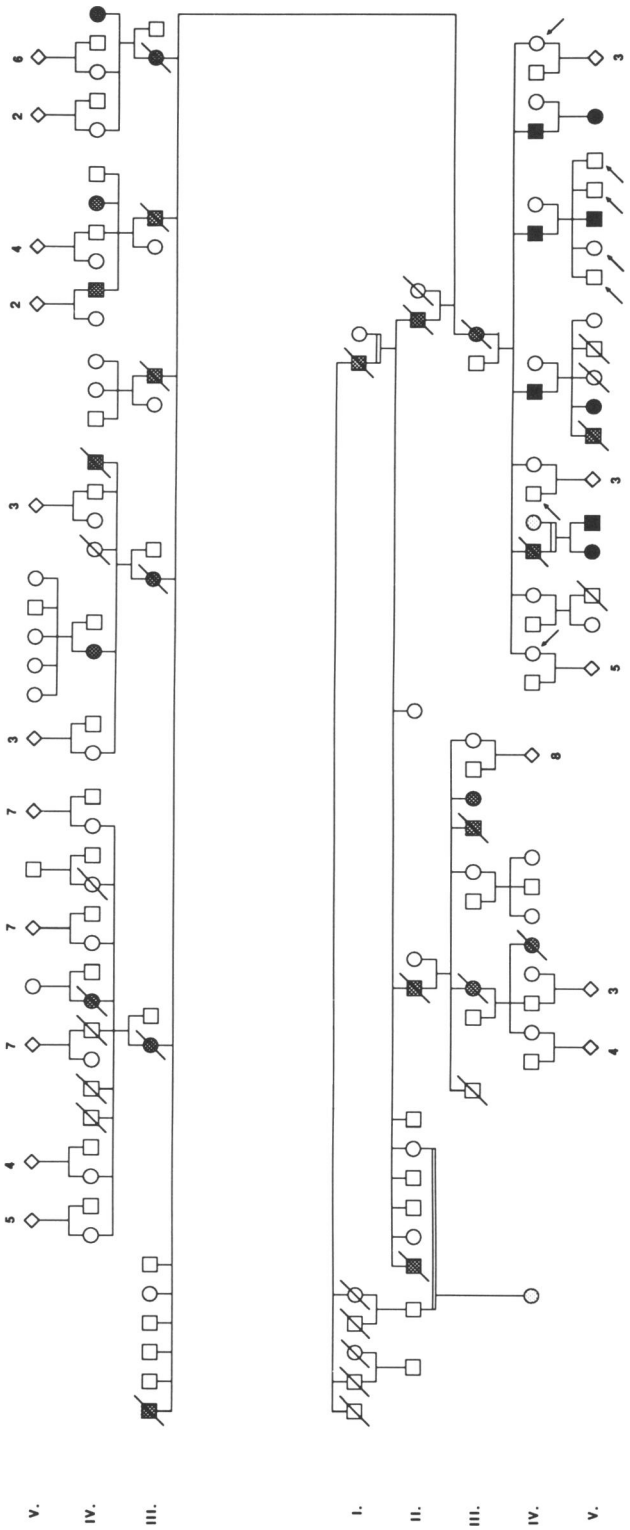


Figure 4 Pedigree OF

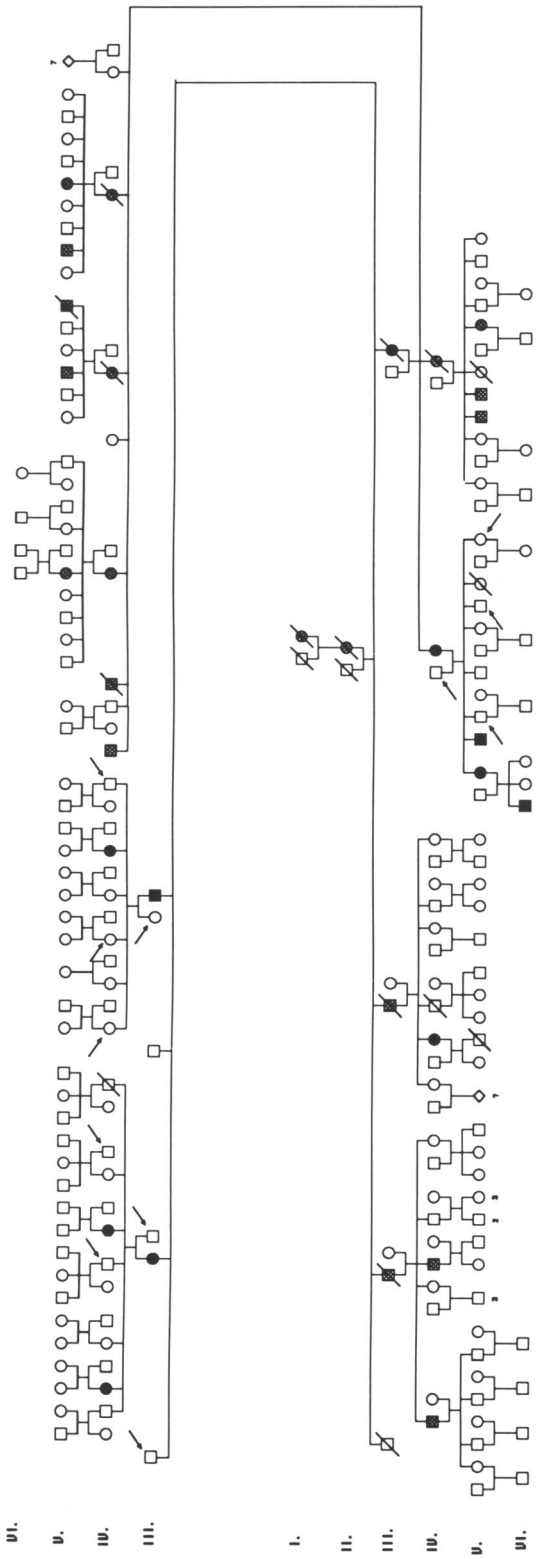


Figure 5 Pedigree Es

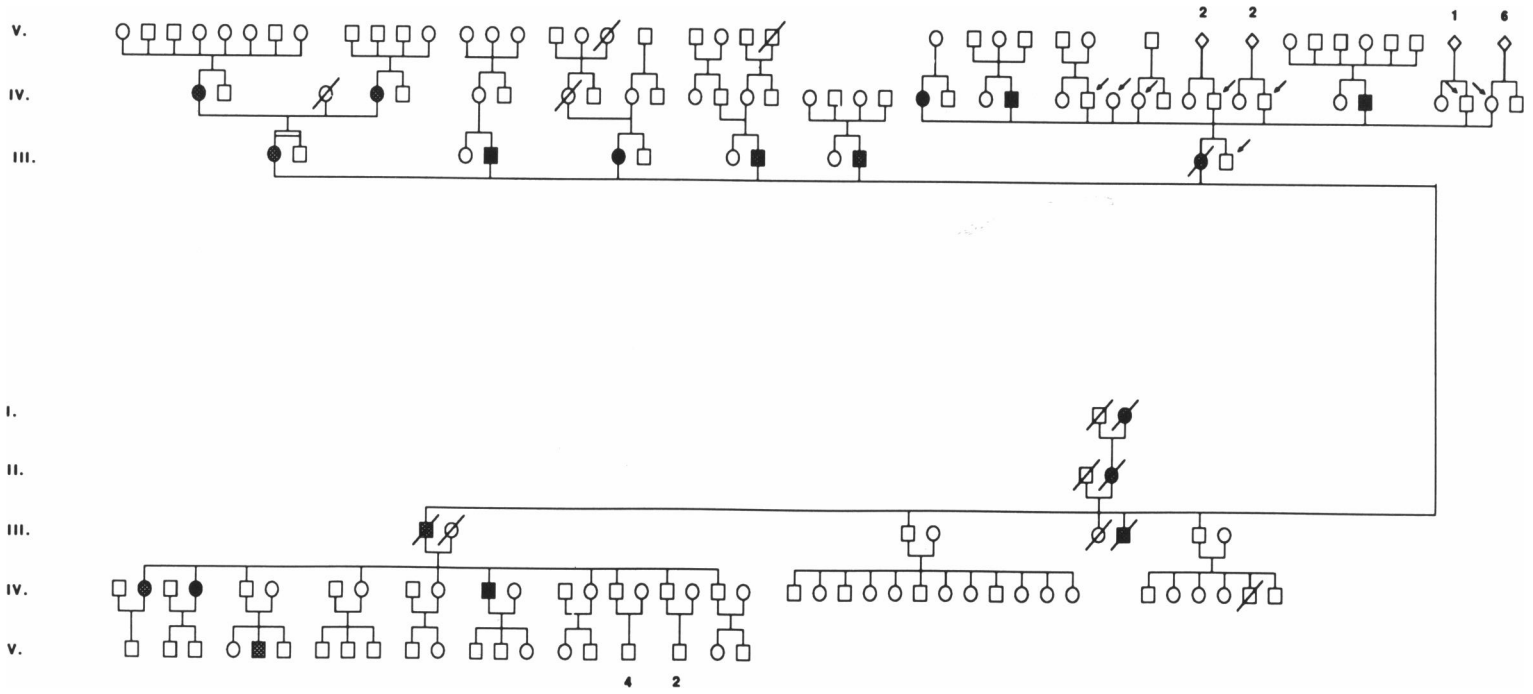


Figure 6 Pedigree In

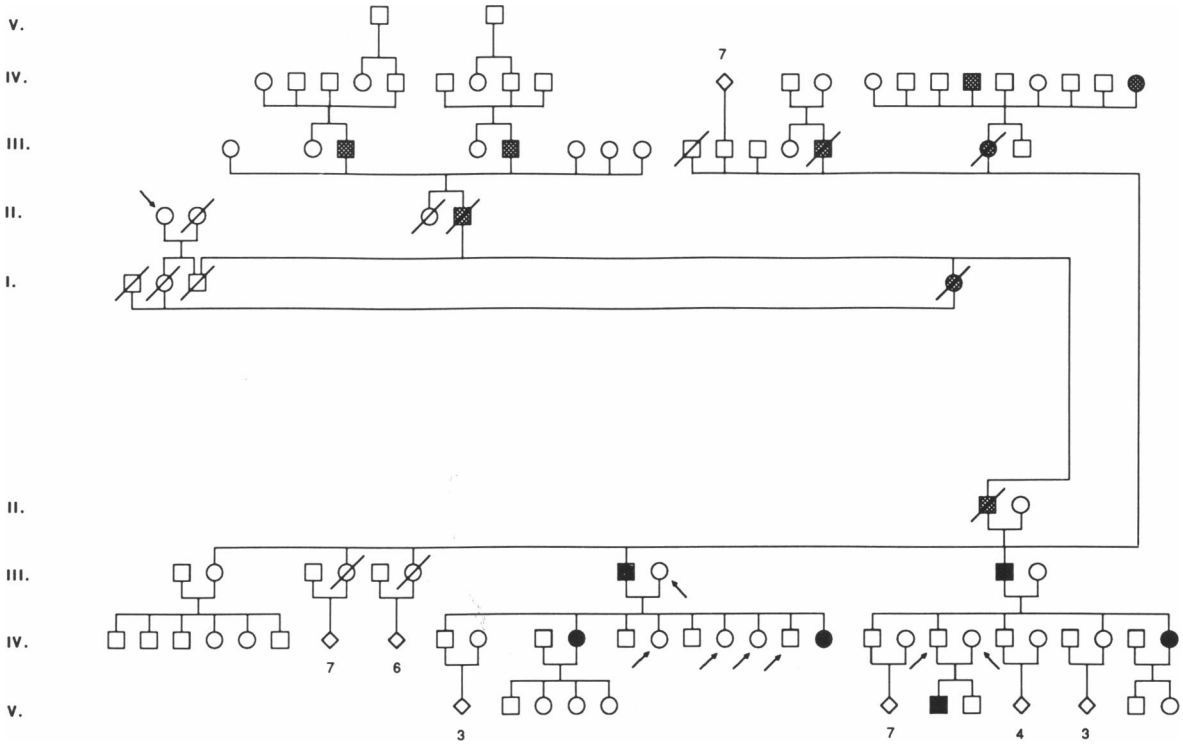


Figure 7 Pedigree Su

enzyme for each polymorphic probe. The resulting DNA fragments were separated by electrophoresis on 0.8% agarose horizontal gels and, after denaturation and neutralization, transferred to a nylon membrane (Hybond-N or Hybond-N+; Amersham PLC) by the method of Southern blotting. The probe inserts were radiolabeled with ^{32}P by random oligonucleotide priming (Feinberg and Vogelstein 1984). Hybridization and autoradiography were performed as described by Gilliam et al. (1984).

To compensate for the variable age at onset within the pedigrees used in the analysis of the data, asymptomatic individuals were attributed a liability class according to their generation number, since it had proved impossible to obtain the age from quite a few individuals. The estimated probability of having developed the disease at a given age was derived from an age-at-onset curve constructed after clinical investigation of 180 patients (Orozco et al., in press-*b*). The youngest generation was estimated to have a 20% probability of penetrance, with older generations estimated to have a 50%, 80%, 95%, or 99% probability. For the three-point analyses, the distance F13A1-HLADQA was set at 30 cM, the distance HLADQA-GLO1 at 8 cM, and the distance GLO1-D6S4/Mut at 21 cM, according to Spence et al. (1989).

Results

Pairwise lod scores between the disease locus and the individual marker loci are summarized in table 2, on the basis of a disease frequency of 1/1,000 and an equal male:female recombination fraction. Exclusion of the disease locus from close proximity to individual loci was established by the generation of a lod score of < -2.0 (Ott 1979).

Simultaneous analysis of more than three loci was not technically feasible with our computational facilities, because of the size of the pedigrees in association with the multiple allelic characteristics of the HLA-DQA and F13A1 loci. Three-point likelihood calculations, when there was an assumption of no interference, yielded maximal lod scores of -5.3 between F13A1 and HLADQA, -10.9 between HLADQA and GLO1, and -5.1 between GLO1 and D6S45/MUT. Variation of the penetrance values of the liability classes did not

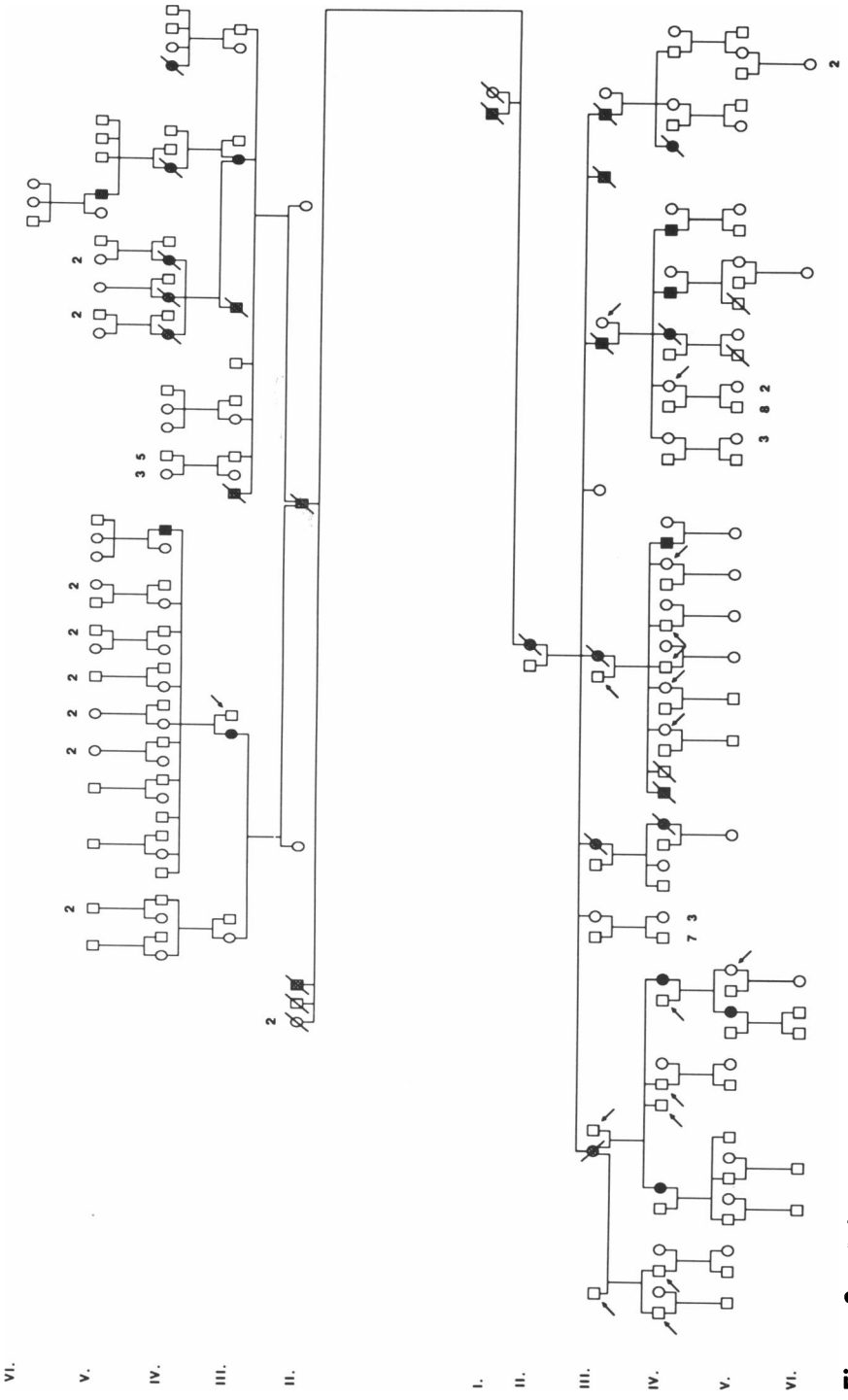
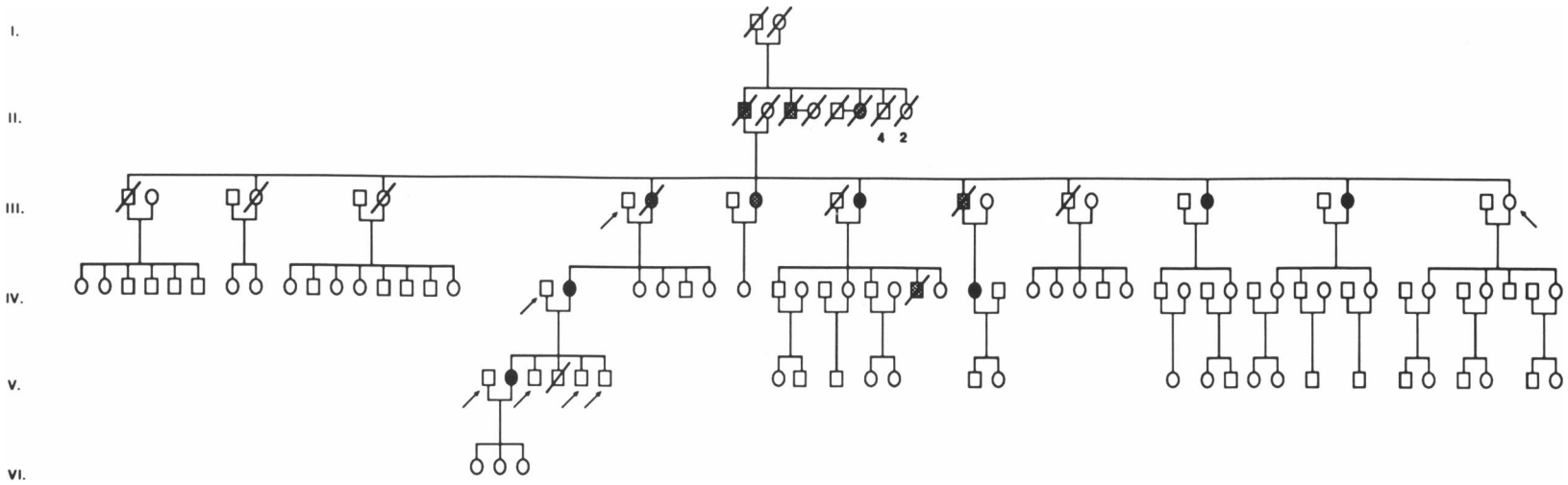


Figure 8 Pedigree Fi



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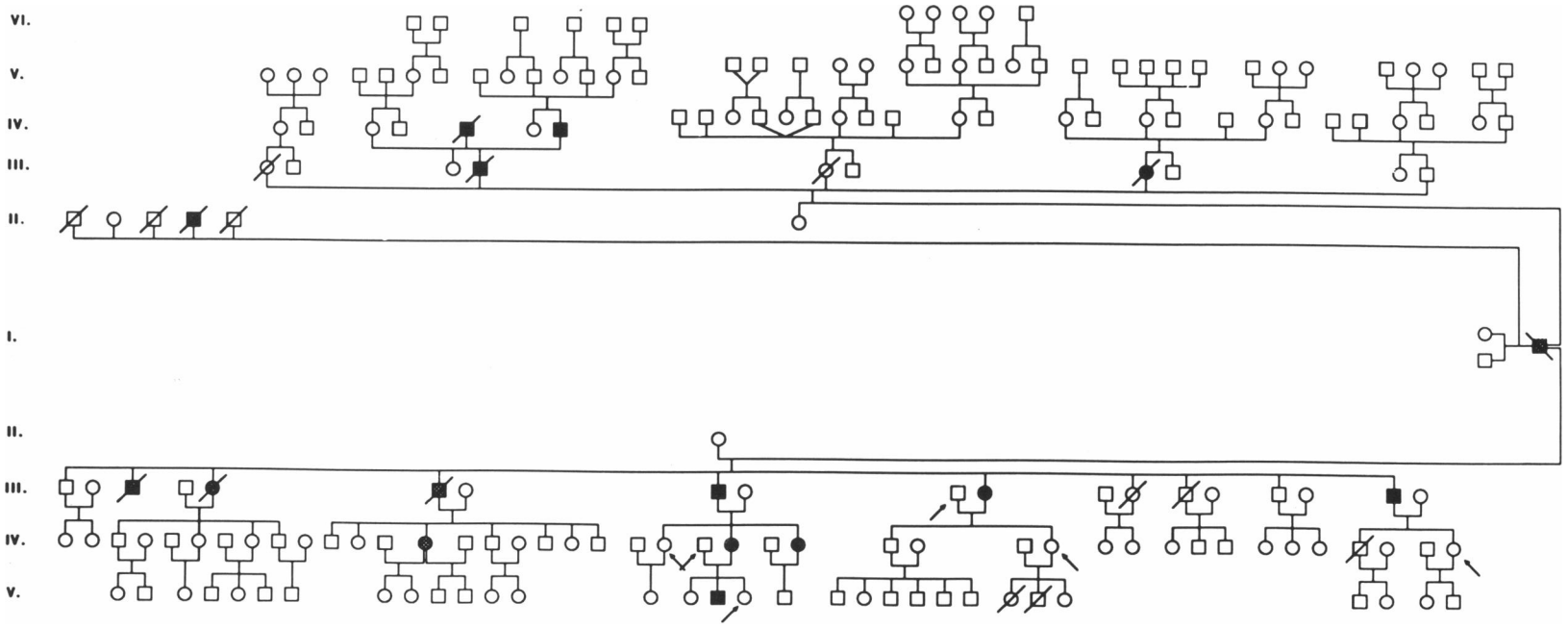

Figure 10 Pedigree Ro

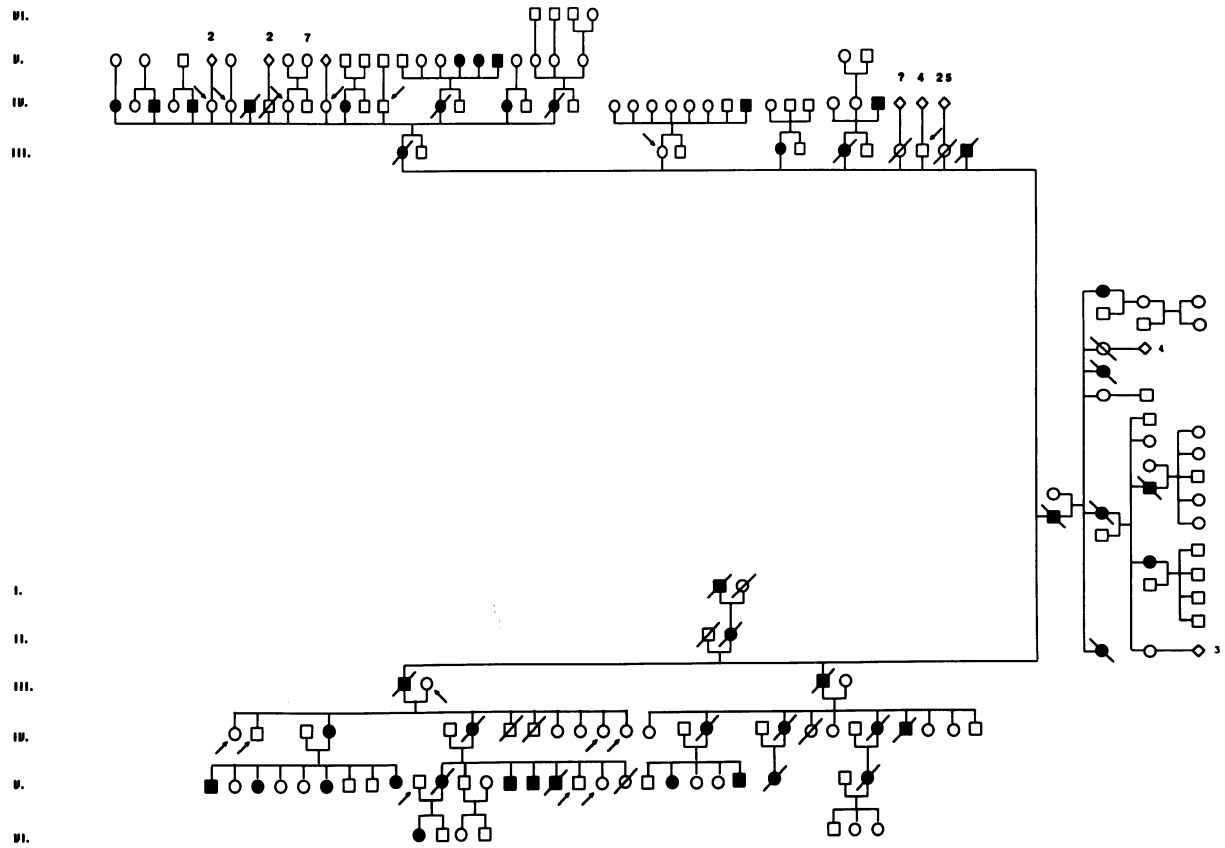

Figure 11 Pedigree Co

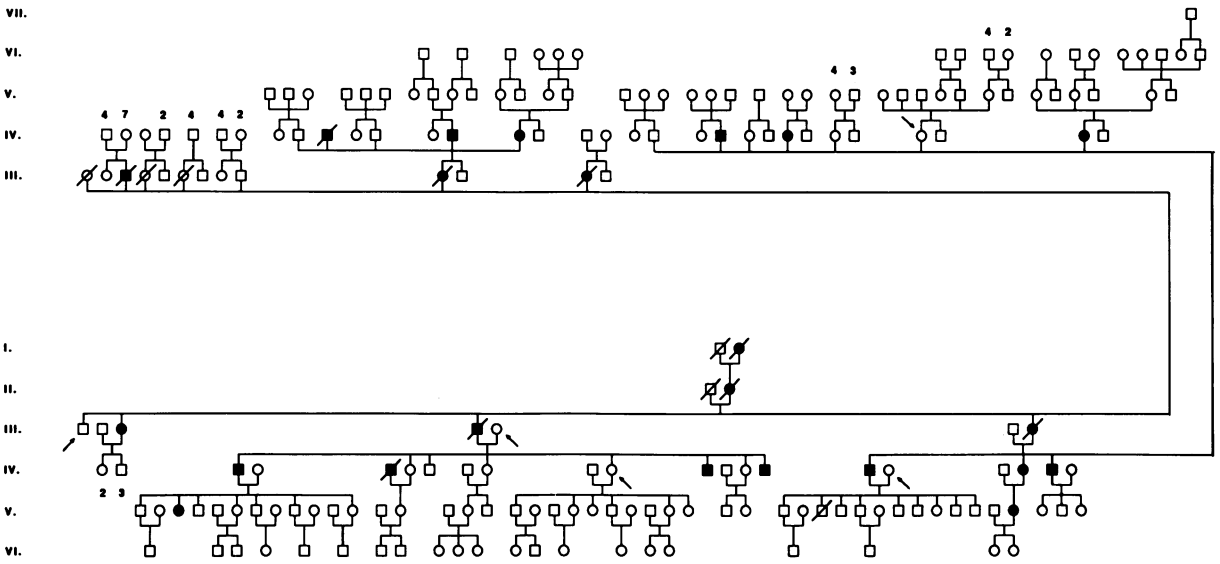

Figure 12 Pedigree Ve

Table 1

Polymorphic Characteristics of Chromosome 6p RFLPs

Probe	Regional Assignment	RFLP Enzyme	Allele Size (kb)	Allele Frequency[a]	Reference
F13A1	6p25-p24	*Bcl*I	13.0	.05	Zoghbi et al. 1988*a*
			12.0	.00	
			11.5	.10	
			10.5	.05	
			9.8	.20	
			9.0	.15	
			8.8	.35	
			8.4	.10	
			6.5	.00	
		*Bam*HI A	13	.90	
			11.5	.10	
		*Bam*HI B	5.5	.10	
			2.9	.90	
HLA DQalpha	6p21.3	*Taq*I	7.4	.18	Bidwell 1988
			6.7	.25	
			5.8	.25	
			4.8	.14	
			2.7	.18	
MUT	6p21	*Hin*dIII	4.3	.60	Zoghbi et al. 1988*b*
			1.8	.40	
D6S4	6p21	*Bgl*II	6.8	.65	Zoghbi et al. 1988*b*
			.62	.35	

[a] As assessed in the population of Holguin.

change the results significantly, and even an analysis scoring only the affected family members still generated the exclusion. The result also held true when it was assumed that future assessment of the genetic distances would find them to differ greatly from the present values. The results have been combined into an interval exclusion map and are summarized in figure 14.

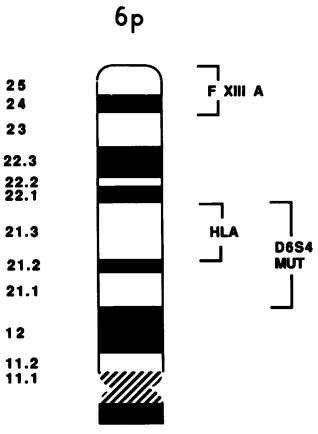

Figure 13 Physical assignment of chromosome 6p RFLP loci according to Spence et al. (1989).

These data exclude the disease locus, in the Cuban pedigrees, from a male/female combined genetic interval of 96 cM on the short arm of chromosome 6, an interval extending 42 cM distally and 54 cM proximally from the HLA locus. This is a conservative estimate, since interference of chiasmata formation during meiosis was not taken into account. The assumption of sex difference in recombination does not alter the result.

Discussion

Autosomal dominant ataxia has been assumed to be heterogeneous on clinical and pathological grounds. Similar claims had been made for the recessively inherited Friedreich ataxia, where genetic locus homogeneity has been subsequently demonstrated (Chamberlain et al. 1989). For the dominant ataxias, it is unclear whether the variability is due to intragenic, intergenic, or polygenic mutations.

Linkage was initially demonstrated to the HLA complex on chromosome 6p in some families with autosomal dominant ataxia (Yakura et al. 1974; Jackson et al. 1977). Considerable efforts have since been made to locate precisely the disease locus in this region. It

Table 2

Lod Scores between Chromosome 6p Markers and Autosomal Dominant Cerebellar Ataxia in Families from Holguin

	Lod Score at Recombination Fraction of						
Probe	.00	.05	.10	.15	.20	.30	.40
F13A1	−14.43	−7.71	−5.09	−3.37	−2.19	−.83	−.23
HLA DQA	−16.92	−7.02	−4.35	−2.72	−1.61	−.39	.05
GLO1	−12.67	−5.96	−3.89	−2.58	−1.68	−.59	−.09
MUT	−5.40	−1.46	−.55	−.13	.06	.10	.00
D6S4	−5.53	−3.05	−1.66	−.79	−.27	.17	.16

is surprising that linkage studies using markers flanking the HLA locus in SCA1 families of almost identical clinical phenotype have revealed the possibility of two alternative locations for the disease locus on chromosome 6p—i.e., either proximal (Zoghbi et al. 1989) or distal (Rich and Orr 1989) to the HLA locus. In each study, the pairwise lod scores and multipoint linkage analysis satisfy statistical criteria.

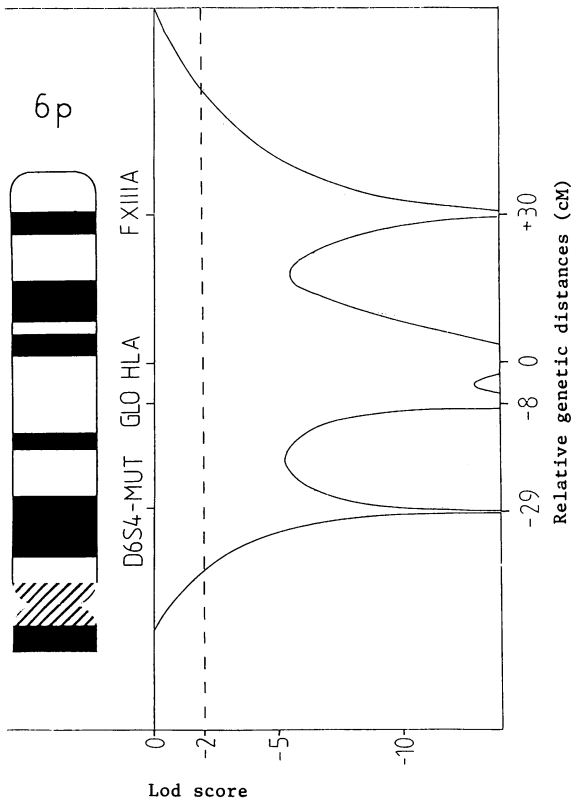

Figure 14 Combination of three-point analyses to an exclusion interval map around four chromosome 6p loci demonstrating the exclusion of the Holguin ataxia mutation from the SCA1 loci.

The identification and subsequent analysis of a large founder-effect population of Holguin patients with dominant ataxia/OPCA was expected to facilitate both the close definition of one SCA1 locus and subsequent identification of the gene. However, analysis of the linkage data from the Cuban pedigrees excluded the disease locus, not only from both proposed SCA1 loci but also from the hypothetical locus of Kumar et al. (1986). These data provide conclusive evidence of heterogeneity within a clinically homogeneous subgroup of dominant ataxia/OPCA, demonstrating that mutations in at least two genes can result in the same clinical picture.

Since no effective therapy is known, the 300 patients already receiving medical attention constitute a severe problem for the regional health authorities in Holguin. While the size of the families will in future allow rapid scientific progress toward localizing the gene and defining therapy, initial investigation has to concentrate on a genomic search to assign the disease locus and thus provide the means for presymptomatic and prenatal diagnosis.

Acknowledgments

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