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Genetic Recombination Events Which Position the Friedreich Ataxia Locus Proximal to the D9S15/D9S5 Linkage Group on Chromosome 9q

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Summary

The absence of recombination between the mutation causing Friedreich ataxia and the two loci which originally assigned the disease locus to chromosome 9 has slowed attempts to isolate and characterize the genetic defect underlying this neurodegenerative disorder. A proximity of less than 1 cM to the linkage group has been proved by the generation of high maximal lod score (Z) to each of the two tightly linked markers D9S15 (Z = 96.69; recombination fraction [θ] = .01) and D9S5 (Z = 98.22; θ = .01). We report here recombination events which indicate that the FRDA locus is located centromeric to the D9S15/D9S5 linkage group, with the most probable order being cen–FRDA–D9S5–D9S15–qter. However, orientation of the markers with respect to the centromere, critical to the positional cloning strategy, remains to be resolved definitively.

Introduction

Friedreich ataxia is a progressive neurodegenerative disorder affecting both the central and peripheral nervous systems. The primary lesion is thought to occur in the dorsal root ganglia, where selective loss of the large myelinated neurons is observed. The incidence of this recessively inherited disorder is estimated to be 1/50,000 in the United Kingdom, with a carrier frequency of 1/110.

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The disease characteristicially presents with ataxia of gait, between the ages of 8 and 12 years. Hypo- or areflexia and small-sensory-nerve action potentials are usually present before symptoms develop. Progressive ataxia, dysarthria, and loss of vibration-and-position sense follow, with patients losing the ability to walk, on average, 15 years after onset. Hypertrophic cardiomyopathy is thought to occur in more than 90% of patients and is the major cause of death.

We have previously mapped the Friedreich ataxia disease locus (FRDA) by linkage analysis to chromosome 9 (Chamberlain et al. 1988; Fujita et al. 1989*a*). A more precise location, 9q13–21.1, immediately distal to the variable heterochromatin region 9qh, was defined by in situ hybridization studies using the two most closely linked markers, MCT112 (D9S15) (Shaw et al. 1990) and DR47 (D9S5) (Fujita et al. 1989*b*).

We subsequently constructed a long-range physical map of the region and identified 10 CpG-rich regions within the 1.7-Mb interval thought to contain the FRDA locus. The two marker loci were shown to lie in close proximity, by the identification of a common 460-kb *Not*I fragment (Fujita et al. 1991; Wilkes et al. 1991).

The D9S15 and D9S5 loci have remained the most closely linked markers to date; no recombination events between FRDA and either marker (or between the markers themselves) had been detected during the analysis of more than 200 multiply affected pedigrees collected worldwide. Attempts were therefore made to increase informativity at the marker loci, in order to detect rare useful recombination events which would orientate the linkage group and position the disease mutation precisely.

Increased informativity at the D9S15 locus was achieved by the identification of both an additional polymorphism detected by *Acc*I (Wallis and Nakamura 1989*a*) and a hypervariable microsatellite sequence (MS) (Fujita et al. 1990; Wallis et al. 1990). More recently, two additional microsatellite sequences, GS4 and GS2, have been identified which flank the D9S15 locus at 90 kb and 60 kb, respectively (Fujita et al. 1992). The D9S5 locus had proved to be extremely uninformative (PIC = .18). Physical expansion of this locus resulted in the isolation of the clone 26P, which detects three RFLPs defined by the enzymes *Bst*XI, *Xba*I, and *Dra*I (Fujita et al. 1990), substantially increasing informativity.

Physical mapping studies in both genomic and yeast artificial chromosome clones (Fujita et al. 1991; Wilkes et al. 1991) have shown the order of these loci within the linkage group to be GS4–MCT112/MS–MCT112/*Msp*I–GS2–26P–DR47. The strength of the linkage data would suggest a proximity for the disease locus of no more than 1 cM from the linkage group, by the lod -1 confidence interval approach. At this proximity, linkage disequilibrium might be observed, hence providing information on the location of the disease locus. Disequilibrium, predominantly with D9S15, has been reported in populations with a potential founder effect, but the association has proved much weaker in several more heterogeneous populations studied.

We now report evidence of genetic recombination events which position the disease locus relative to the two closest markers. These data are discussed in relation to the clinical phenotypes and the potential problems with definitive diagnosis when one is working at this level of resolution. The relevance of disequilibrium as an indicator for proximity is reviewed.

Subjects and Methods

Subjects

Families accepted for inclusion in this analysis conformed to the basic diagnostic criteria for Friedreich ataxia as defined by Geoffroy et al. (1976) and Harding (1981), with two exceptions—(1) an absolute requirement that the age at onset be before 20 years of age and (2) the presence of typical electrocardiographic changes (Harding and Langton-Hewer 1983) in at least one affected member of each family. Genetic linkage analysis was carried out in five centers and included 274 families with two or more affected individuals: St. Mary's Hospital Medical School, London (SM) (104 families); Laboratoire de Genetique Moleculaire, Strasbourg (LGM) (46 families); Hôpital Ste. Justine, Montreal (SJ) (35 families); Instituto Nazionale Neurologico "Carlo Besta," Milan and Universita di Napoli (CB-UN) (58 families); and Hospital "La Fe," Valencia (LF) (31 families). This heterogeneous group of families is predominantly European or North American, although some patients were of Asian or northern African origin.

Linkage Analysis

Linkage analysis between the FRDA locus and the polymorphic loci, D9S15 and D9S5, was carried out in all families. After the detection of recombination, positioning of the crossover events with respect to the D9S15/D9S5 linkage group was investigated by including in the analysis the highly polymorphic microsatellite loci, GS4 and GS2 (table 1), known to flank D9S15 at 90 kb and 60 kb, respectively.

Lod score (Z) values were computed for combined sexes (recombination fraction [θ] for males [θ_m] = θ for females [θ_f]), as tests for a sex difference in recombination were insignificant in both pairwise and multipoint analysis. To establish a multipoint linkage map of proximal 9q and in an attempt to orientate the linkage group, the SM pedigrees were also analyzed with polymorphic loci previously shown to flank the Friedreich ataxia region—i.e., D9S48 (9q11) and ALDH1 (9q21.1) (Chamberlain et al. 1989*b*) (table 1).

Pairwise Z values were calculated, using the LINKAGE computer program (Lathrop et al. 1985), between FRDA and (*a*) the individual polymorphic sys-

Table 1

Polymorphic Characteristics of Chromosome 9q Marker Loci

Locus	Probe	Regional Assignment	Enzyme	PIC	Source of Data
D9S48	MCT7	9q11	*Pst*I	. . .	Y. Nakamura, personal communication
D9S15	MCT112	9q13-21.1	*Msp*I	.33	Carlson 1987
			*Acc*I	.34	Wallis and Nakamura 1989
D9S5	MS			.75	Wallis et al. 1990
	DR47	9q13-21.1	*Taq*I	.18	Orzechowski et al. 1987
ALDH1	26P		*Bst*XI	.55	Fujita et al. 1990
		9q21.2	*Msp*I	.33	Yoshida and Chen 1989

	Length of Amplified DNA (bp)	PCR Primers
GS2	124	Direct: 5′AATGAAATAGAATTTCACAGG 3′ Reverse: 5′AACCCTTCTGTCAGACAAGGA 3′
GS4	131	Direct: 5′GGGAAGAGCAAATTCCCTGAACCCCG 3′ Reverse: 5′CCTGGGCGACAGAGTGAGACTCG 3′

tems; (*b*) the compound haplotype at the D9S15 locus (MS/ / MCT112/*Msp*I), where the polymorphic sites are known to lie within an interval of no more than 5 kb; and (*c*) the D9S5 locus (DR47/*Taq*I/ /26P/ *Bst*XI), where the sites lie within 30 kb. Multipoint analysis was carried out in the SM pedigrees, over the genetic interval containing the linkage group D9S48–[D9S15–D9S5]–ALDH1.

Linkage Disequilibrium

Linkage disequilibrium studies between FRDA and the marker loci focused on D9S15 and D9S5. Association studies for the individual loci and for the extended haplotypes in the French (Fujita et al. 1990), Italian (Pandolfo et al. 1990), French-Canadian (Richter et al. 1990), and Spanish (Monrós et al., submitted) patient populations have been published or submitted for publication. In the St. Mary's pedigree resource, allelic association was investigated for individual polymorphisms and for the haplotype at the D9S15 (MS/ / MCT112/*Msp*I) locus. Although these loci lie in close physical proximity, association with the extended haplotype D9S15/D9S5 was not investigated in this instance, because of the recombination observed in this region.

Clinical Evaluation of Recombinant Families

The clinical features observed in the affected members of the recombinant families are described in table 2. Consanguinity was present in family LF2 only.

In all cases, onset occurred before the age of 20 years, the disease commonly presenting as general unsteadiness. Affected members have progressive ataxia accompanied by dysarthria, posterior column signs, and diminished or absent tendon reflexes. Cardiomyopathy, considered to be a primary feature of the disorder, was present in at least one affected member of each family.

Of the six recombinant families, two (SM1 and SM2) are from England, two (LF1 and LF2) are from Spain, one (SJ1) is from Canada, and one (CB-UN1) is from Italy. Clinical diagnosis in these families has been reviewed and confirmed by a neurologist from each group.

Family SM1.—This is a nuclear pedigree with two of the three offspring affected. Age at onset and rate of progression vary considerably between the sibs, the female sib becoming severely disabled and confined to a wheelchair at about the age of puberty. The male sib remained ambulant until the age of 22 years.

Family SM2.—This is a nuclear pedigree with two of the three offspring affected. Despite a very similar age at onset in both sibs, the rate of progression has varied. While one sib became confined to a wheelchair at the age of 25 years, the sister could still walk unaided for considerable distances, until approximately age 35 years. It is surprising that evidence of heart disease could not be detected in the more severely affected sib when last examined, while individual II.1 had an abnormal electrocardiogram.

Table 2

Clinical Features of the Six Recombinant Pedigrees, Indicating the Primary Diagnostic Criteria

	Family SM1		Family SM2		Family LF1		Family LF2			Family SJ1		Family CB-UN1	
	II.1	II.2	II.1	II.2	II.1	II.2	II.2	II.6	II.7	II.2	II.3	II.1	II.2
Age (years)	23	18	50	38	20	14	28	20	18	35	31	25	24
Sex	M	F	F	F	F	F	F	M	M	F	F	M	F
Age at onset (years)	13	7	16	13	5	7	12	10	12	4	4	14	13
Ataxia	+	+	+	+	+	+	+	+	+	+	+	+	+
Dysarthria	+	+	+	+	+	+	+	+	+	+	+	+	±
Tendon reflexes	−	−	−	−	−	−	−	−	−	−	−	−	−
Position and vibration sense	−	−	−	−	−	−	−	−	−	−	−	−	−
Electrocardiogram	A	A	A	N	A	NT	A	A	A	A	A	A	A
Diabetes	N	N	NT	NT	N	N	N	N	N	+	+	N	N

NOTE.— + = Present; − = diminished or absent; A = abnormal; N = normal; and NT = not tested.

Family LF1.—This is a nuclear pedigree with two offspring, both affected.

Family LF2.—This is a nuclear pedigree with three of seven offspring affected. Enlargement of the fourth ventricle and vermis atrophy in individual II.3 and an anomalous morphology of the fourth ventricle in II.6 were detected on computed-tomography scan. The parents are third cousins.

Family SJ1.—This is a family with two of four offspring affected. Age at onset was comparatively early (about 4 years of age), but otherwise the features were unremarkable. Abnormal glucose tolerance has been detected in the affected sibs. The unaffected sister has been investigated extensively within the past 6 mo, and normal nerve conduction velocities (median, 56 m/s; peroneal, 53 m/s) have been recorded.

Family CB-UN1.—This is a nuclear pedigree with both offspring affected. Enlargement of the supra- and infratentorial subarachnoid space and fourth ventricle was detected on computed-tomography scan after a disease duration of 11 years. Nerve biopsy showed loss of large myelinated fibers.

Results

Linkage Analysis

Combined pairwise Z values between the FRDA locus and chromosome 9q markers are summarized in table 3. Data generated from the analysis of the SJ, LGM, CB-UN, and LF pedigrees with the markers D9S15 and D9S5 have been published (in part) elsewhere (Richter et al. 1989; Fujita et al. 1990; Pandolfo et al. 1990).

Maximal Z values were generated between the FRDA locus and D9S15 ($Z = 96.69$; $\theta = .01$) and D9S5 ($Z = 98.22$; $\theta = .01$), reflecting the detection of recombination events for each marker locus. These θ's should be regarded as good estimates, since the lod table is somewhat sparse around the maximum. In addition, analysis of the SM and LF pedigree sets revealed recombination between the D9S15 and D9S5 loci ($\theta = .02$).

The D9S48 locus remains closely linked to FRDA ($Z = 11.2$; $\theta = .06$), the disparity between the genetic and the estimated physical distance (greater than 10 Mb) in this region being maintained. In contrast, analysis of this extended pedigree set resulted in a weakening of the linkage between FRDA and the ALDH1 locus ($Z = 3.75$; $\theta = .12$).

Recombination Events

Recombination events were detected between the disease locus and one or more of the marker loci in six of the families analyzed. Figure 1 summarizes the analysis in these families for the markers D9S48 (MCT7/*Pst*I), GS4, D9S15 (MCT112/MS), D9S15 (MCT112/*Msp*I), GS2, D9S5 (26P/*Bst*XI), D9S5 (DR47/*Taq*I), and (if informative) ALDH1.

In each of the families SM1, SM2, and LF2, the crossover event appears to be maternal in origin. In families SM1 and SM2, recombination was detected at each locus informative in the respective mothers: family SM1 at D9S15 (MCT112/*Msp*I) and ALDH1; and family

Table 3

Pairwise Z between Chromosome 9q Markers and FRDA

Locus	Z AT θ							
	.00	.001	.010	.050	.100	.200	.300	.400
D9S15:[a]								
SM	−99.99	35.77	41.51	40.74	35.74	23.80	12.27	3.47
LGM	25.89	25.83	25.26	22.74	19.52	12.95	6.71	1.89
SJ[b]	13.70	13.62	13.30	11.80	9.90	6.30	3.20	.87
CB-UN	13.95	13.92	13.60	12.15	10.30	6.64	3.35	.93
LF	−99.99	.28	3.02	3.72	3.43	2.36	1.22	.35
Overall	−99.99	89.42	96.69	91.15	78.89	52.05	26.75	7.51
D9S5:[c]								
SM	−99.99	27.95	32.91	32.45	28.45	18.92	9.77	2.77
LGM	27.89	27.81	27.16	24.25	20.57	13.32	6.74	1.86
SJ[d]	−99.99	18.33	18.40	17.00	14.70	9.80	5.20	1.32
CB-UN	11.45	11.42	11.15	9.95	8.47	5.60	2.96	.87
LF	8.82	8.72	8.60	7.68	6.54	4.31	2.24	.63
Overall	−99.99	94.23	98.22	91.33	78.73	51.95	26.91	7.45
Additionial loci analyzed in SM pedigrees only:								
D9S48	−99.99	1.839	8.360	11.145	10.614	7.494	3.956	1.129
ALDH1	−99.99	−10.853	−2.164	2.740	3.718	3.101	1.731	.509

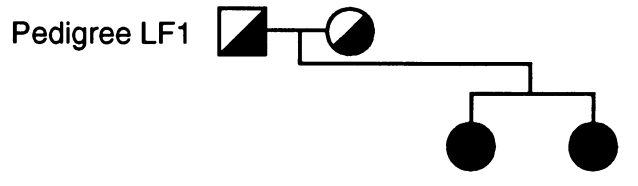
[a] Data are combined Z values for (MCT112/*Msp*I//MS) haplotype.
[b] Z values are generated from the analysis of MCT112/*Msp*I.
[c] Data are combined Z values for (26P/*Bst*XI//DR47/*Taq*I) haplotype.
[d] Z values are generated from the analysis of 26P/*Bst*XI.

SM2 at GS4, D9S15 (MCT112/MS), D9S5 (26P/*Bst*XI), and ALDH1. In family LF2 recombination was detected at GS4 and D9S15 (MCT112/MS) but not at 26P. The crossover event in family LF1 is paternal in origin, with recombination detected at each locus informative in the father: GS4, D9S15 (MCT112/MS) / (MCT112/*Msp*I), and GS2.

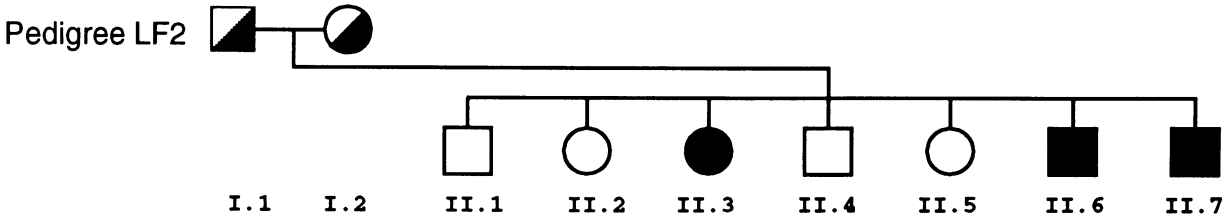
Full informativity was observed at the GS4, MCT112/MS, and 26P/*Bst*XI loci in family SJ1. Identical paternal and maternal haplotypes were inherited both by the two affected sibs and by their normal sister. The significance of recombination detected in a normal individual is always tenuous, because of the possibility of that individual developing the disease at a later date. In this family, onset was comparatively early (approximately 4 years of age). Concordance for age at onset within a sibship is generally 5 years or less. Consequently, as this individual is now 23 years old, the probability that she will develop Friedreich ataxia in the future is unlikely. This is corroborated by the failure to detect the earliest symptoms of this disorder, despite repeat investigations carried out during the past 3 years.

Family CB-UN1 was initially thought to be recombinant for GS4. Chromosomal phase could not be established, because of the absence of genotype data from the deceased mother (I.2). However, analysis of her living father and sibs facilitated haplotype reconstruction in this individual (full data available on request). This subsequently revealed that one allele of the GS4 microsatellite marker in individual II.2 could not have been inherited from either parent. Mendelian inheritance of the highly informative tetranucleotide repeat 4F7 (chromosome 11) and several other equally informative markers demonstrated that nonpaternity was extremely unlikely in this family. Consequently, the presence of this new allele must represent a mutation, at the GS4 locus, which transformed allele 8 into allele 7 by the addition of a single repeat unit—probably the result of replication slippage. The gamete in which this occurred could not be determined, since individual II.2 has inherited identical extended haplotypes from both parents.

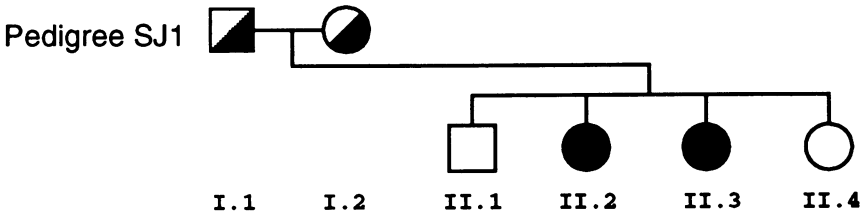
In summary, these recombination events clearly exclude the disease locus from the interval defined by the GS4–D9S15–GS2–D9S5 linkage group which



	I.1		I.2		II.1		II.2	
GS4	1	3	2	4	3	4	1	4
112/MS	3	6	5	3	6	3	3	3
112/Msp	2	1	1	2	1	2	2	2
GS2	2	4	3	3	4	3	2	3
26P	2	2	2	1	2	1	2	1
DR47	2	2	1	2	2	2	2	2
ALDH1	1	1	2	1	1	1	1	1



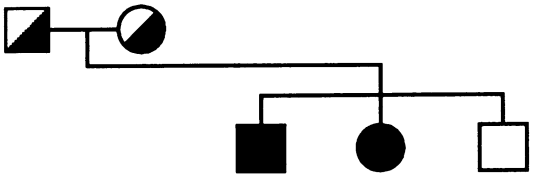
	I.1		I.2		II.1		II.2		II.3		II.4		II.5		II.6		II.7	
GS4	2	2	1	2	2	1	2	1	2	1	2	1	2	2	2	2	2	2
112/MS	3	6	5	6	3	5	6	5	6	5	6	5	3	6	6	6	6	6
112/Msp	2	1	1	1	2	1	1	1	1	1	1	1	2	1	1	1	1	1
GS2	1	2	2	2	1	2	2	2	2	2	2	2	1	2	2	2	2	2
26P	1	2	3	1	1	3	2	3	2	1	2	3	1	1	2	1	2	1
DR47	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
ALDH1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1



	I.1		I.2		II.1		II.2		II.3		II.4	
GS4	13	12	7	9	7	12	13	9	13	9	13	9
112/MS	3	1	2	3	2	1	3	3	3	3	3	3
112/Msp	1	2	1	1	1	2	1	1	1	1	1	1
GS2	4	2	4	4	4	2	4	4	4	4	4	4
26P	2	3	1	3	1	3	2	3	2	3	2	3
DR47	2	2	2	2	2	2	2	2	2	2	2	2

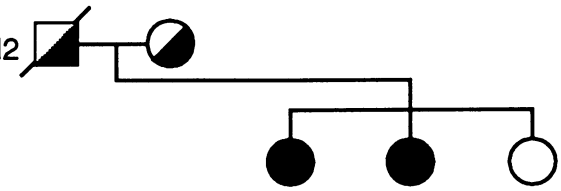
Figure 1 Genetic analysis of the six Friedreich ataxia pedigrees in which recombination was detected between the disease locus and one or more of the markers constituting the D9S48–[GS4–D9S15–GS2–D9S5]–ALDH1 linkage group. The loci 112/MS and 112/*Msp*I constitute D9S15. 26P and DR47 constitute the locus D9S5. Allele sizes (bp) for the 112/MS locus are MS1 = 209; MS2 = 207; MS3 = 205; MS4 = 203; MS5 = 201; MS6 = 199; MS7 = 197; and MS8 = 195. The allele sizes do not compare directly with those reported

Pedigree SM1



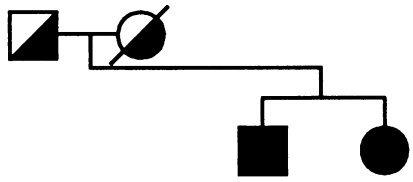
	I.1		I.2		II.1		II.2		II.3	
MCT 7	2	1	2	2	2	2	2	2	1	2
GS4	6	12	9	9	6	9	6	9	12	9
112/MS	5	6	3	3	5	3	5	3	6	3
112/Msp	1	1	1	2	1	1	1	2	1	2
GS2	4	2	3	3	4	3	4	3	2	3
26P	2	3	2	2	2	2	2	2	3	2
DR47	2	2	2	2	2	2	2	2	2	2
ALDH1	2	2	1	2	2	1	2	2	2	2

Pedigree SM2



	I.1*		I.2		II.1		II.2		II.3	
MCT 7	2	1	2	2	1	2	1	2	2	2
GS4	6	9	6	12	9	6	9	12	6	6
112/MS	6	7	6	7	7	6	7	7	6	6
112/Msp	1	1	1	1	1	1	1	1	1	1
GS2	4	4	4	4	4	4	4	4	4	4
26P	2	3	3	1	3	3	3	1	2	3
DR47	2	2	2	2	2	2	2	2	2	2
ALDH1	2	2	2	1	2	2	2	1	2	2

Pedigree CB-UN1



	I.1		I.2*		II.1		II.2	
MCT 7	2	2	2	2	2	2	2	2
GS4	8	9	4/13	8	8	8	8	7
112/MS	6	5	2	6	6	6	6	6
112/Msp	1	1	1	1	1	1	1	1
GS2	3	4	4	3	3	3	3	3
26P	3	3	3	3	3	3	3	3
DR47	2	2	2	2	2	2	2	2
ALDH1	2	2	2	2	2	2	2	2

by Fujita et al. (1990), as allele MS1 (209 bp) was not observed by these authors—MS2 = M1; MS3 = M2; etc. Reference allele sizes for GS4 and GS2 are allele 13 = 131 bp and allele 4 = 124 bp, respectively (Sirugo et al. 1992). In family CB-UN1, the genotype of individual I.2 at GS4 is either 4,8 or 13,8. The non-Mendelian inheritance of allele 7 in individual II.2 (*shaded*) is probably indicative of replication slippage. An asterisk denotes that haplotypes were inferred.

spans approximately 550 kb of proximal 9q. These data, in association with recombination events observed between the disease locus and additional distal 9q markers including ALDH1, position FRDA centromeric to the linkage group. Interpretation of the analysis in family LF2, where recombination is detected between FRDA and the marker loci GS4 and D9S15 (MCT112/MS) but, despite full informativity, not at D9S5 (26P/*Bst*XI), implies that the most probable order is cen–FRDA–D9S5–D9S15–qter.

Multipoint Analysis

The multipoint genetic map of the region, as based on marker-marker recombination events, was constructed using the data set generated from the SM pedigrees, both including and excluding the recombinant families. Zero recombination was assumed between the two polymorphic loci (MCT112/MS and MCT112/*Msp*I) at D9S15 and also between the two loci (26P/*Bst*XI and DR47/*Taq*I) at D9S5. The genetic distances were subsequently computed by iterating the three θ's between the markers. The resulting genetic map of the linkage group was entirely consistent with the physical mapping order: cen–D9S48–(.16)–[D9S15–(.02)–D9S5]–(.14)–ALDH1. For both data sets, the order D9S48–D9S15–D9S5–ALDH1 was favored by odds of only 9.6:1 over the alternative order D9S48–D9S5–D9S15–ALDH1.

Linkage Disequilibrium

Association studies in the French-Canadian (SJ) (Richter et al. 1989), northern-Spanish (SM) (Chamberlain et al. 1989*a*), French (LGM) (Fujita et al. 1990), and Italian (CB-UN) (Pandolfo et al. 1990) patient populations have been reported elsewhere. In summary, no statistically significant evidence of association between FRDA and the D9S5 locus has been published for any patient population except the French (Fujita et al. 1990), while significant linkage disequilibrium has been reported between the disease and D9S15—in particular, the 205-bp allele of the MCT112/MS polymorphism (Hanauer et al. 1990*a*). These data implied a closer proximity of the FRDA locus to D9S15 than to D9S5.

Disequilibrium studies in the SM pedigree set were carried out between the FRDA locus and the individual polymorphisms and the haplotypes generated at the D9S15 locus. The analysis of allelic association between FRDA and the DR47/*Taq*I polymorphism at D9S5 was excluded because of low informativity in these families.

As the SM pedigrees constitute families collected in collaboration throughout the world, the possibility of disequilibrium was investigated separately in (*a*) the U.K. pedigrees ($n = 41$) and (*b*) the complete pedigree resource. As both data sets are comparatively small, the families were not subdivided further according to geographical origin. No evidence of allelic association between the FRDA locus and the microsatellite polymorphism (i.e., MS) at D9S15, the *Msp*I polymorphism at D9S15, the *Bst*XI polymorphism at D9S5, or the haplotype (MCT112/MS//MCT112/*Msp*I) at D9S15 was found (data available on request).

Discussion

The isolation and subsequent characterization of a disease locus depends on the construction of physical and genetic maps of the linked region and on the positioning of genetic recombination events in relation to the physical location of potential "candidate" coding sequences. Progress toward the isolation of the mutation causing Friedreich ataxia has, ironically, been slowed by the tight linkage between FRDA and two anonymous markers MCT112 (D9S15) and DR47 (D9S5), preventing ordering within the linkage group. However, the tight linkage has facilitated the provision of reliable genetic counseling in affected families (Wallis et al. 1989; Hanauer et al. 1990*b*).

We now report recombination events between FRDA and the closest markers, detected after the identification of more highly informative polymorphisms. Combined maximal Z values between FRDA and D9S15 and FRDA and D9S5 are $Z = 96.69$ ($\theta = .01$) and $Z = 98.22$ ($\theta = .01$), respectively. Using the confidence interval approach, linkage analysis provides firm evidence that the FRDA locus lies within 1 cM of the linkage group; the recombination data indicate a position for the disease locus centromeric to the D9S15/D9S5 linkage group on proximal 9q. The critical issue remains the orientation of the linkage group with respect to the centromere, which in turn determines the directional cloning strategy.

This could not be resolved by multipoint linkage analysis; the probability of the order cen–D9S15–D9S5–ALDH1 is only approximately 10 times more likely than the order cen–D9S5–D9S15–ALDH1. Only the analysis of family LF2 provides evidence for order indicating that D9S5 and, hence, FRDA lie centromeric to D9S15—consistent with the evidence for order generated by extended haplotype analysis of the Cajun population (Sirugo et al. 1992). However, as

the parents are third-degree cousins, homozygosity would be predicted at each of the informative marker loci in the affected individuals. As homozygosity is lost at the 26P locus, a recombinant event in a former meiosis can therefore be deduced.

Corroborating evidence from disequilibrium profiles across the region has been sought. The analysis of the SM pedigrees provides no evidence for linkage disequilibrium. In addition, while previous association studies have demonstrated significant disequilibrium only between FRDA and D9S15 (Fujita et al. 1990; Pandolfo et al. 1990), recent extension of the French, Italian, and French-Canadian pedigree sets has resulted in the detection of statistically significant disequilibrium at D9S5, with a concomitant diminishing degree of association with D9S15 (M. Koenig, M. Pandolfo, and A. Richter, unpublished data). This shift in the disequilibrium profile could be the result of the inclusion of affected pedigrees from more heterogeneous populations, as exemplified in the Spanish population. Strong disequilibrium with D9S5 was initially observed from the study of families originating in the immediate vicinity of Valencia. Extension of the study to include families collected throughout Spain has subsequently resulted in the loss of significant disequilibrium at either locus.

However, this is still a comparatively small data set. In addition, we can only speculate on the number of different mutations which can cause Friedreich ataxia. In view of the variation in age at onset, rate of progression, and severity, more than one mutation would be predicted. These factors, singly or in combination, could explain the absence of linkage disequilibrium in this particular patient group. In summary, support for locus order based on linkage disequilibrium is ambiguous, although it would seem (from unpublished data) that association between FRDA and D9S5 (26P/*Bst*XI) is at least as significant as that seen between FRDA and D9S15, in certain populations.

The inclusion of GS4, a microsatellite polymorphism, for genetic counseling and in disequilibrium studies is questionable, after the detection of probable replication slippage in family CB-UN1. This type of polymorphism, with a large number of alleles, may be evolving rapidly by mechanisms other than meiotic crossover (Levinson and Gutman 1987; Wolff et al. 1988). It would seem likely that certain microsatellite polymorphisms, including GS4, may not share the disequilibrium relationships found for conventional RFLPs. Consequently, data including such loci must be interpreted with caution.

The possibility of heterogeneity must always be considered when any genetic disorder is analyzed, particularly if there is marked clinical variation. In the case of Friedreich ataxia, locus homogeneity has been conclusively demonstrated in all patient populations conforming to the basic diagnostic criteria used here, and we regard accurate diagnosis as central to this study. Furthermore, analysis of these data by using the HOMOG program revealed no evidence of heterogeneity.

However, all groups have encountered cases with clinical phenotypes initially indistinguishable from Friedreich ataxia. Following the detection of recombination across the entire D9S48–D9S15–D9S5–ALDH1 region in one family, further investigation showed a vitamin E deficiency (M. Pandolfo, unpublished data). In addition, several "recombinant" families in which the only deviation from the classical criteria is the absence of cardiomyopathy, despite long disease duration, also show exclusion from the chromosome 9 locus (S. Chamberlain and A. Harding, unpublished data).

It therefore appears that the presence of heart disease is critical for diagnosis of Friedreich ataxia with a locus on 9q. However, the age at which this pathology becomes evident varies even within families (Harding and Langton-Hewer 1983). In the Cajun patient population reported by Keats et al. (1989), where the disease phenotype and course are more benign, cardiomyopathy is a less prominent feature of the disorder, with increased longevity of affected members.

For the six families reported here, clinical notes have been reviewed by clinicians from each research group, and the diagnosis has been confirmed. Resampling has also been undertaken to eliminate laboratory error. As inversion of the 9qh region is common, with an incidence of 1%, cytogenetic analysis of family LF2 has been undertaken. No evidence of pericentric inversion has been detected, supporting the proposed order for the marker loci. Taking all data into consideration, we propose that the most likely gene/probe order in this region is cen–FRDA–D9S5–GS2–D9S15–GS4–qter.

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