Am. J. Hum. Genet. 47:228–235, 1990

Friedreich Ataxia in Italian Families: Genetic Homogeneity and Linkage Disequilibrium with the Marker Loci D9S5 and D9S15

Massimo Pandolfo,* Giorgio Sirugo,‡ Antonella Antonelli,* Leonor Weitnauer,* Luca Ferretti,† Maurizio Leone,§ Ivano Dones,* Antonella Cerino,* Ricardo Fujita,‡ Andre Hanauer,‡ Jean-Louis Mandel,‡ and Stefano Di Donato*

*Istituto Neurologico "C. Besta," and †I.D.V.G.A.—C.N.R., Milan; ‡INSERM U.184, Strasbourg; and §Ospedale Civile di Ivrea, Ivrea, Italy

Summary

Friedreich ataxia (FA) is an autosomal recessive degenerative disease of the nervous system of unknown biochemical cause. The FA gene has been shown to be in close linkage with the two chromosome 9 markers D9S5 and D9S15, and linkage disequilibrium between FA and D9S15 has been detected in French families by Hanauer et al. We used new highly informative markers at the above loci to analyze Italian FA families for linkage and linkage disequilibrium. The new markers were a three-allele *Bst*XI RFLP at D9S5 (PIC = .55) and a six-allele microsatellite, typed by polymerase chain reaction, at D9S15 (PIC = .75). We obtained maximum lod scores of 8.25 between FA and D9S5, 10.55 between FA and D9S15, and 9.52 between D9S5 and D9S15, all at zero recombination. Our results, combined with those reported by other authors, reduce maxlod-1 (maximum lod score minus 1) confidence limits to less than 1.1 cM between FA and D9S5, 1.2 cM between FA and D9S15, and 1.4 cM between D9S5 and D9S15. Linkage disequilibrium with FA was found only for D9S15 when all families were evaluated but was also found for a D9S5/D9S15 haplotype in a subgroup of southern Italian families. We conclude that FA, D9S5, and D9S15 are tightly clustered and that studies of geographically restricted groups may reveal a limited number of mutations responsible for the disease in the Italian population. We present preliminary evidence from pulsed-field gel electrophoresis that D9S5 and D9S15 may be less than 450 kb apart. Linkage disequilibrium between FA and D9S15 suggests that the disease gene may be at an even shorter distance from this marker locus, which therefore represents a very good starting point for cloning attempts.

Introduction

Friedreich ataxia (FA) is an autosomal recessive progressive neurodegenerative disorder, characterized by selective loss of large myelinated fibers in the dorsal roots and by degeneration of the spinocerebellar tracts. Criteria for diagnosis, as defined by Geoffroy et al. (1976) and by Harding (1981), include onset before 25 years of age, progressive ataxia of trunk and limbs, loss of tendon reflexes in lower limbs, posterior column signs in lower limbs, extensor plantar responses, and cardiomyopathy. Pes cavus, kyphoscoliosis, nystagmus, distal amyotrophy, and dysarthria are common accessory signs. Progression leads to confinement in a wheelchair within a few years. In Italy the disease has an estimated incidence of 1/25,000 (Romeo et al. 1983). Despite extensive biochemical studies, the basic defect of FA is still elusive, and nothing is known about the nature of the disease gene. Chamberlain et al. (1988) mapped the FA gene on chromosome 9 by genetic linkage with the polymorphic locus D9S15, recognized by the probe MCT112 (Carlson et al. 1987). Shortly after, Fujita et al. (1989) described linkage between FA and another chromosome 9 marker, D9S5, recognized by the probe DR47 (Orzechowski et al. 1987). In these and later studies (Chamberlain et al. 1989; Hanauer et al. 1990), no recombination was detected between FA, D9S5, and D9S15 in all informative meioses examined. Fujita et al. (in press) recently found a single crossover between FA and D9S15, but, because of the family structure,

Received December 7, 1989; final revision received April 5, 1990.

Address for correspondence and reprints: Massimo Pandolfo, Divisione di Biochimica e Genetica del Sistema Nervoso, Istituto Neurologico "C. Besta," Via Celoria 11, 20133 Milano, Italy.

0002-9297/90/4702-0007$02.00

it was not possible to establish whether it also involved the D9S5 locus. Linkage disequilibrium between D9S15 and FA has been shown by Hanauer et al. (1990). Physical mapping by in situ hybridization of D9S5, performed by Hanauer et al. (1990) and by Raimondi et al. (in press), assigned the D9S5/D9S15/FA group of loci to bands 9q12-q21.1. New polymorphisms at the D9S5 and D9S15 loci have recently been described (Fujita et al., in press). These polymorphisms increase substantially the number of families informative for linkage analysis, and they can be used to construct extended haplotypes for the evaluation of linkage disequilibrium. In the present study we analyzed 29 Italian FA families for linkage and linkage disequilibrium with D9S5 and D9S15.

Material and Methods

Families

We studied a total of 29 Italian FA families. Fourteen families had two to four affected children, while the remaining families had only one affected child with unaffected sibs. In order to define the geographic origin of our families, we recorded the place of birth of all parents and, whenever possible, of grandparents. This was necessary because internal migrations in the past 30 years make it unreliable to determine geographic origin solely on the basis of current place of residence. Twelve parents were born in southern Italy, four of them in Sicily and eight in the southeastern Abruzzo and Apulia regions. All remaining parents were from northern Italian regions. Pedigree structures, phenotypic data, and age-at-onset information are available from the authors on request.

Clinical Evaluation

All affected and unaffected subjects whose blood was obtained for linkage analysis were examined neurologically by at least one of the authors, either at the Istituto Neurologico "C. Besta," (Milan) or at the Division of Neurology, Hospital of Ivrea (Ivrea, Italy). The diagnosis of FA was established by strict criteria (Geoffroy et al. 1976; Harding 1981). All affected persons were thoroughly investigated with a number of instrumental

Table 1

DNA Polymorphisms Analyzed in FA Families

A. RFLPs

Locus	Probe	Enzyme	Constant Band (kb)	Allele			PIC
				Symbol	Size (kb)	Frequency	
D9S5	DR47	*Taq*I		1	5.0	.92	.14
				2	4.0	.08	
		*Msp*I		1	4.3	.92	.14
				2	2.7	.08	
	26P	*Bst*XI	2.5	1	5.0	.20	.55
				2	4.0 + 1.0	.31	
				3	3.5 + 1.5	.49	
D9S15	MCT112S[a]	*Msp*I		1	6.0	.71	.33
				2	4.9	.29	

B. MS

Locus	Oligonucleotide for Amplification	Allele			PIC
		Symbol	Size Difference (bp)	Frequency	
D9S15.....	MCT112D: 5′-CCCTCAGGTCTATTGAAGAAGTATAAAT-3′	1	+10	.08	.75
		2	+8	.16	
		3	+6	.08	
		4	+4	.24	
	MCT112R: 5′-GATGGTGGTAATCATTTCACAG-3′	5	+2	.36	
		6	0	.08	

[a] Contains a 1.9-kb *Sph*I fragment of MCT112 devoid of repetitive sequences.

and biochemical techniques, including CT scan, EEG, EMG, electrocardiography, echocardiography, and glucose- and pyruvate-tolerance tests. Some of the patients from both institutions have been described in detail elsewhere (D'Angelo et al. 1980; Leone et al. 1988).

Molecular Analysis

Genomic DNA was isolated from leukocytes according to the standard technique, except that protein salting out was substituted for phenol extraction (Miller et al. 1988). Each individual was typed for the polymorphisms shown in table 1. Two new polymorphisms were included in our analysis: a three-allele *Bst*XI RFLP detected by the probe 26P at D9S5 and a six-allele microsatellite polymorphism at D9S15. The former was found in a search for more informative RFLPs at D9S5, which utilized fragments from two overlapping cosmid clones spanning 48 kb around the region recognized by the probe DR47, and the latter was directly identified by sequencing the probe MCT112 (Fujita et al., in press).

For RFLP analysis, 10 μg of genomic DNA were digested with the appropriate restriction enzyme, electrophoresed on 0.8% agarose gels, and transferred onto nylon membranes (GeneScreen Plus™; DuPont) by alkaline blotting. Gel-purified inserts were labeled with α^{32}P-dNTPs by the random oligonucleotide primer method. Blot hybridization and washing were done according to the membrane manufacturer's instructions. Autoradiography was on Kodak X-AR films at −70°C for 24–48 h. The microsatellite polymorphism was typed by amplification of the corresponding segment of genomic DNA (about 160 bp) in a polymerase chain reaction (PCR). The two oligonucleotides shown in table 1 were used as primers. One of the oligonucleotides (MCT112D) was end-labeled with ^{32}P in a reaction containing 200 pmol primer, 40 pmol γ^{32}P-ATP at 5,000 Ci/mmol, 10 mM $MgCl_2$, 5 mM DTT, 50 mM Tris-HCl (pH 7.6), and 10 units T4 polynucleotide kinase. PCRs were carried out in a Perkin Elmer temperature cycler with 1 μl genomic DNA used as template (~200 ng), 2.5 μl end-labeled primer reaction, 3 pmol cold reverse primer (oligonucleotide MCT112R), 200 μM of each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.2), 3 mM $MgCl_2$, and 1 unit *Taq* polymerase (Perkin Elmer–Cetus), in a final volume of 20 μl. DNA was amplified through 25 temperature cycles, each consisting of 1 min at 92°C for denaturation, 45 s at 65°C for annealing, and 1 min at 72°C for extension. One and one-half microliters of each amplification reaction were loaded on 8% polyacrylamide-urea sequencing gels and were run at 1,500 V for 4 h. Gels were exposed to Kodak X-AR films for 2 h at room temperature.

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis was performed on a Pharmacia-LKB Pulsaphor apparatus, using the contour clamped homogeneous field (CHEF) technique. Lymphocytes from a normal subject were included in low-gelling-temperature agarose plugs at 2×10^6 cells/ml. DNA purification and restriction-endonuclease digestions were done according to methods described in the literature (Smith and Cantor 1986). Plugs were loaded on 1% agarose gels in 1 × TBE, and CHEF was run for 24 h at 160 V with pulses of 30–60 s in different experiments. DNA was blotted on nylon membranes by the alkaline transfer method, including a depurination step of 15 min in 0.25 M HCl. Hybridization with probes MCT112S and 26P was as described for RFLP analysis.

Statistical Analysis

The LINKAGE package of programs (Lathrop et al. 1985) was used for two- and multipoint linkage analysis. Pairwise lod-score tables were obtained both between each marker locus and FA and between marker loci, by using the MLINK program. Locus order and maximum-likelihood estimates of recombination fractions were obtained using the ILINK program.

Linkage disequilibrium was evaluated by the χ^2 test, and Yates's correction was used when appropriate. Significance levels were corrected for multiple comparisons as follows: $\alpha' = 1 - (1-\alpha)1/k$, where α is the risk of type I error chosen as significance level (e.g., .05), k is the number of comparisons, and α' is the corrected significance level. The standardized disequilibrium coefficient (Δ) was calculated according to the method of Chakravarti et al. (1984).

Results

Linkage Analysis

Linkage analysis results are summarized in table 2. In accordance with previous reports by other groups, we found the loci D9S15 and D9S5 to be in close linkage with FA.

Fourteen families were at least partially informative for the *Msp*I RFLP (M) detected by the probe MCT112 at D9S15; one family was partially informative for the M and *Taq*I RFLPs detected by the probe DR47 at D9S5;

Table 2

Linkage Analysis Results

A. Results from Present Study							
	Pairwise Lod Score at Recombination Fraction of						
Loci	.00	.001	.05	.1	.2	.3	.4
FA-D9S5[a]	8.25	8.23	7.27	6.27	4.23	2.28	.68
Fa-D9S15[b]	10.55	10.52	9.20	7.83	5.11	2.61	.73
D9S5-D9S15[c]	9.52	9.50	8.54	7.52	5.37	3.13	1.03
B. Combined Data: Results from Present and Other Studies							
	Pairwise Lod Score at Recombination Fraction of						
Loci	.00	.005	.01	.02	.03	.04	.05
FA-D9S5[d]	36.12	35.76	35.20	34.28	33.36	32.43	31.49
FA-D9S15[e]	$-\infty$	58.37	57.99	56.71	55.34	53.87	52.40
D9S5-D9S15[d]	35.69	35.32	34.95	34.22	33.47	32.72	31.95

[a] Combined analysis of B and M at D9S5.
[b] Combined analysis of M and microsatellite polymorphism at D9S15.
[c] Combined analysis of B and M at D9S5, and of M and microsatellite polymorphism at D9S15.
[d] Data are from present study and Fujita et al. (1990).
[e] Data are from Chamberlain et al. (1989) and Fujita et al. (1990).

and all families were informative both for the *Bst*XI RFLP (B) detected by the probe 26P at D9S5 and for the microsatellite polymorphism (MS) at D9S15. When *Msp*I/ *Bst*XI haplotypes were at D9S5 and when *Msp*I/microsatellite haplotypes were used at D9S15 in the analysis, maximum lod scores were 8.25 between FA and D9S5 and 10.55 between FA and D9S15, both at zero recombination. A maximum lod score of 9.52 was obtained between D9S5 and D9S15, and again no crossovers were detected. No recombination was found in three-point analysis also, so no information about gene order could be obtained.

When our results are combined with those of Fujita et al. (1990), which include those reported in Hanauer et al. (1990), the maximum lod score between FA and D9S5 becomes 36.12 at zero recombination, reducing the maxlod-1 (maximum lod score minus 1) confidence interval to less than 1.1 cM. A similar combination of our data both with those of Chamberlain et al. (1989) and with those of Fujita et al. (1990), gives a maximum lod score between FA and D9S15 of 58.37 at a recombination fraction of .005, with a maxlod-1 confidence limit of less than 1.2 cM. The combined maximum lod score between D9S5 and D9S15 is 35.69 at zero recombination, with a maxlod-1 confidence limit narrowed to less than 1.4 cM.

Linkage Disequilibrium between Polymorphisms at D9S15

The six MS alleles and the two M alleles form 12 possible haplotypes at the locus D9S15. We found all these haplotypes among 102 typed parental chromosomes from our FA families. Strong linkage disequilibrium was present between MS alleles and M al-

Table 3

Distribution of M and MS Alleles at D9S15

	No. (%) of M Alleles	
MS Allele	1	2
1[a]	2 (2.8)	8 (25.8)
2[a]	6 (8.4)	19 (61.3)
3	7 (9.9)	1 (3.2)
4[b]	24 (33.8)	1 (3.2)
5[b]	28 (39.4)	1 (3.2)
6	4 (5.6)	1 (3.2)
Total	71	31

Note.—Six comparisons were carried out in this table, giving an $\alpha' = .0085$ for $\alpha = .05$. The χ^2 for the whole table is 52.95 with 5 df ($P << .001$).
[a] Associated with M 2 ($P < .005$).
[b] Associated with M 1 ($P < .005$).

leles: MS 1 and 2 were preferentially associated with M 2, and MS 4 and 5 were preferentially associated with M 1 ($P < .005$) (table 3).

Linkage Disequilibrium between D9S5 and D9S15

No significant linkage disequilibrium was found between B alleles at D9S5 and M alleles at D9S15.

Linkage Disequilibrium between FA and the Marker Loci

We could type 52 FA chromosomes and 50 normal chromosomes for D9S15 and could type 51 FA chromosomes and 44 normal chromosomes for D9S5. The slightly lower number of chromosomes typed for D9S5 was due only to the limited amount of DNA available from some individuals, and in no case was the determination of the alleles associated with the disease equivocal.

At D9S5, only the B detected by the probe 26P was evaluated, because of the very low frequency of the rare alleles of the other polymorphisms. As shown in table 4, we found allele B 3 in slight excess on chromosomes not carrying the FA mutation and found allele B 2 on FA chromosomes, but the differences did not reach statistical significance.

Conversely, linkage disequilibrium between D9S15 and FA was present at a statistically significant level, with overrepresentation of allele M 2 (table 5). Δ was .22. The excess of M2 on FA chromosomes was reflected in a higher frequency of MS-M haplotypes 1-2 and 2-2, although linkage disequilibrium could not be established, at a statistically significant level, with either of these haplotypes.

We constructed extended haplotypes by putting together B alleles at D9S5 with MS and M alleles at D9S15. The observed haplotype distribution is shown in table 6: we found in our sample 22 of the 36 possible B-MS-M extended haplotypes, 11 of which were represented in more than 5% of either normal or FA chromosomes. With regard to the frequency of single haplotypes, we found that, between chromosomes carrying the FA mutation and normal chromosomes, there was no difference that was statistically significant at the levels required for multiple comparisons; nevertheless, some remarks can be made about the distribution of haplotypes in the two groups. There were eight haplotypes represented on more than 5% of FA chromosomes: these accounted for 72.5% of FA chromosomes but for only 43.2% of chromosomes not carrying the FA mutation. Conversely, five haplotypes were present on more than 5% of chromosomes not carrying the FA mutation, accounting for 59% of them, but these five haplotypes accounted for only 29.4% of FA chromosomes, and only two extended haplotypes (3-4-1 and 1-5-1) were present on at least 5% of both normal and FA chromosomes. The two most represented extended haplotypes on FA chromosomes were (B-MS-M) 2-4-1 (15.7%) and 2-2-2 (11.8%). Both were less common (2.3% and 4.5%, respectively) on normal chromosomes. The excess of 2-4-1 was significant when chromosomes of persons from southern Italy were considered (table 6*B*). Haplotype 3-5-1, on the contrary, was more common

Table 4

Frequencies of B Alleles at D9S5 on Normal and FA Chromosomes

B Allele	No. (%) of Normal Chromosomes	No. (%) of FA Chromosomes
1	7 (15.9)	10 (19.6)
2	13 (29.5)	23 (45.1)
3	24 (54.5)	18 (35.3)
Total	44	51

Table 5

Frequencies of M Alleles and MS-M Haplotypes at D9S15 on Normal and FA Chromosomes

A. Frequency of M Alleles[a]

M Allele	No. (%) of Normal Chromosomes	No. (%) of FA Chromosomes
1	40 (80.0)	31 (59.6)
2	10 (20.0)	21 (40.4)
Total	50	52

B. Frequency of MS-M Haplotypes

Alleles		No. (%) of Normal Chromosomes	No. (%) of FA Chromosomes
MS	M		
1	1	2 (4.0)	0
1	2	2 (4.0)	6 (11.5)
2	1	3 (6.0)	3 (5.8)
2	2	5 (10.0)	14 (26.9)
3	1	5 (8.0)	3 (5.8)
3	2	0	1 (1.9)
4	1	11 (22.0)	13 (25.0)
4	2	1 (2.0)	0
5	1	17 (34.0)	11 (21.1)
5	2	1 (2.0)	0
6	1	3 (6.0)	1 (1.9)
6	2	3 (2.0)	0
Total		50	52

[a] $\chi^2 = 5.007$ with 1 df ($P < .05$).

Table 6

Frequencies of D9S5/D9S15 Extended Haplotypes on Normal and FA Chromosomes

A. All Families

Alleles			No. (%) of Normal Chromosomes	No. (%) of FA Chromosomes
B	MS	M		
1	5	1	3 (6.8)	3 (5.9)
1	Others[a]	1	3 (6.8)	1 (2.0)
1	2	2	1 (2.3)	4 (7.8)
1	Others[a]	2	0	2 (3.9)
2	3	1	3 (6.8)	1 (2.0)
2	4	1	1 (2.3)	8 (15.7)
2	5	1	3 (6.8)	5 (9.8)
2	Others[a]	1	3 (6.8)	0
2	2	2	2 (4.5)	6 (11.8)
2	Others[a]	2	1 (2.3)	3 (5.9)
3	2	1	1 (2.3)	3 (5.9)
3	4	1	7 (15.9)	4 (7.8)
3	5	1	10 (22.7)	2 (3.9)
3	Others[a]	1	3 (6.8)	3 (5.9)
3	2	2	1 (2.3)	4 (7.8)
3	Others[a]	2	2 (4.5)	2 (3.9)
Total			44	51

B. Southern Italian Families[b]

Alleles			No. (%) of Normal Chromosomes	No. (%) of FA Chromosomes
B	MS	M		
1	4	1	2 (16.7)	1 (8.3)
2	1	1	2 (16.7)	0
2	4	1[c]	0	6 (50.0)
2	5	1	2 (16.7)	0
2	2	2	0	2 (16.7)
3	5	1	3 (25.0)	0
Other haplotypes			3 (25.0)	3 (25.0)
Total			12	12

[a] Haplotypes represented in less than 5% of both normal and FA chromosomes have been grouped according to the B and M allele.

[b] $\chi^2 = 15.33$ with 6 df ($P \approx .02$).

[c] In excess on FA chromosomes ($P < .05$).

(22.7% vs. 3.9%) on chromosomes not carrying the FA mutation.

Pulsed-Field Gel Electrophoresis

Probes 26P and MCT112S both detected a *Not*I band of 450 kb, as shown in figure 1. Bands detected on restriction digests with *Cla*I, *Mlu*I, *Sal*I, *Nar*I, *Nci*I, *Nru*I, *Sac*II, and *Sma*I, all in the 120–350-kb range, were of different sizes for the two probes (not shown).

Discussion

The linkage data derived from our families confirm and reinforce those already published, further proving the genetic homogeneity of FA and the tight genetic association of D9S5 and D9S15 with the disease locus. We used new and more informative polymorphisms at the marker loci, which resulted in much higher lod scores than would have been obtained by using only

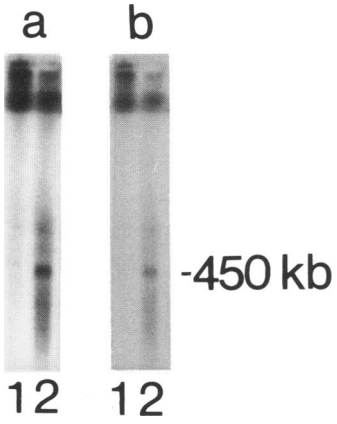

Figure 1 Southern blot of a pulsed-field gel electrophoresis of peripheral lymphocyte DNA from a normal subject. The same blot was hybridized with probe MCT112S (*a*) and with probe 26P (*b*). Lanes 1, Undigested DNA. Lanes 2, *Not*I-digested DNA. An identical band of approximately 450 kb is detected by both probes on *Not*I digests.

the original RFLPs. The microsatellite polymorphism at D9S15 was particularly useful in this regard. It represents an example of a recently discovered class of genetic markers, one that is due to variations in the number of tandem repeats of the CA dinucleotide (Weber and May 1989). Microsatellites may have many alleles, increasing both the chance of heterozygosity and, consequently, the number of informative families for linkage studies. Furthermore, the procedure for typing microsatellites is quicker than the one used for RFLPs, and, thanks to the amplification procedure, it requires only 100–200 ng of genomic DNA. A reasonable objection to their use in genetic studies is the possibility of high mutation rates due to unequal crossing-overs that change the number of CA repeats (Jeffreys et al. 1988). However, at least in our case, the presence of strong linkage disequilibrium between MS and M alleles at D9S15 argues against this occurrence (table 3). It is clear that FA, D9S5, and D9S15 form a tightly linked cluster of loci, likely to be genetically even closer than the maxlod-1 confidence limits indicate, as pointed out by the existence of linkage disequilibrium.

Preferential association of FA with the rare M allele at D9S15 (M 2) has been reported in Quebec families (Richter et al. 1989), where a strong founder effect is present. Hanauer et al. (1990), described the same association in French families, the ethnic group from which Quebec families derived. A further analysis of the French families identified the D9S15 MS-M haplotype 2-2 and the D9S5/D9S15 B-MS-M extended haplotype 2-2-2 as the most preferentially associated with FA (Fujita et al. 1990). In Italian families M 2 is associated with FA at a lower but statistically significant level, while MS-M 2-2 is not so strikingly increased on FA chromosomes as it is in French families, in part because the excess of M 2 seems to be due also to MS-M 1-2 (table 5). Linkage disequilibrium between FA and D9S5 is weaker and is not immediately apparent, but the analysis of D9S5-D9S15 B-MS-M extended haplotypes suggests that it may be actually present. The clearest evidence in this regard is the overrepresentation of B-MS-M 2-4-1 on FA chromosomes: MS-M 4-1 is almost equally as frequent on normal chromosomes, but it is very rarely associated with B 2. Furthermore, this association is geographically limited to southern Italy, where this haplotype was found in six of 12 FA chromosomes (four from southeastern Italy and two from Sicily) and in no chromosome not carrying the FA mutation. On the other hand, the large excess of B-MS-M 2-2-2 found in French families is not present in our sample, in which B 1, 2, and 3 are almost equally distributed on FA chromosomes carrying MS-M 2-2. These data suggest that studies of geographically restricted groups will be necessary to better define linkage disequilibrium between FA and marker loci, even when, as in our study, the families examined are from a single country.

The genetic evidence of tight linkage among FA, D9S5, and D9S15 raises the question about their actual physical distance. Our pulsed-field gel electrophoresis experiments suggest that D9S5 and D9S15 are at a maximum of 450 kb apart. Linkage disequilibrium between these two loci, as between D9S5 and FA, seems to be weaker then that between D9S15 and FA. If this directly reflects physical location, FA should be at an even shorter distance from D9S15 — and therefore within range of the currently available cloning techniques.

Acknowledgments

We thank Drs. Y. Nakamura and A. J. Driesel for probes MCT112 and DR47. This work was supported by the Associazione Italiana per la lotta alle Sindromi Atassiche (AISA) and by a "Progetto finalizzato 1989–1990" of the Italian Ministero della Sanità.

References

Carlson M, Nakamura Y, Krapcho K, Fujimoto E, O'Connell P, Leppert M, Lathrop GM, et al (1987) Isolation and mapping of a polymorphic DNA sequence pMCT112 on chromosome 9q (D9S15). Nucleic Acids Res 15:10614

Chakravarti A, Buetow KH, Antonarakis SE, Waber PG, Boehm CD, Kazazian HH (1984) Nonuniform recombination within the human β-globin gene cluster. Am J Hum Genet 36:1239–1258

Chamberlain S, Shaw J, Rowland A, Wallis J, South S, Nakamura Y, von Gabain, et al (1988) Mapping of mutation causing Friedreich's ataxia to human chromosome 9. Nature 334:248–250

Chamberlain S, Shaw J, Wallis J, Rowland A, Chow L, Farrall M, Keats B, et al (1989) Genetic homogeneity at the Friedreich ataxia locus on chromosome 9. Am J Hum Genet 44:518–521

D'Angelo A, Di Donato S, Crenna G, Negri S, Beulche F, Uziel G, Boeri R (1980) Friedreich's ataxia. I. Clinical, neurophysiological and in vivo biochemical studies. Ital J Neurol Sci 4:231–238

Fujita R, Agid Y, Trouillas P, Seck A, Tommasi-Davenas C, Driesel AJ, Olek K, et al (1989) Confirmation of linkage of Friedreich's ataxia to chromosome 9 and identification of a new closely linked marker. Genomics 4:110–111

Fujita R, Hanauer A, Sirugo G, Heilig R, Mandel JL (1990) New polymorphisms at marker loci D9S5 and D9S15 generate extended haplotypes in linkage disequilibrium with Friedreich's ataxia. Proc Natl Acad Sci USA 87:1796–1800

Geoffroy G, Barbeau A, Breton G, Lemieux B, Aube M, Lepert C, Bouchard RW (1976) Clinical description and roentgenologic evaluation of patient with Friedrich's ataxia. Can J Neurol Sci 3:279–287

Hanauer A, Chery M, Fujita R, Driesel AJ, Gilgenkrantz S, Mandel JL (1990) The Friedreich's 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

Harding AE (1981) Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. Brain 104:589–620

Jeffreys AJ, Royle NJ, Wilson V, Wong Z (1988) Spontaneous mutation rates to new length alleles at tandem repetitive hypervariable loci in human DNA. Nature 332:278–281

Lathrop GM, Lalouel JM, Julier C, Ott J (1985) Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 37:482–498

Leone M, Rocca WA, Rosso MG, Mantel N, Schoenberg BS, Schiffer D (1988) Friedreich's disease: survival analysis in an Italian population. Neurology 38:1433–1438

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Orzechowski HD, Hennig J, Winter P, Grzeschik KH, Olek K, Driesel AJ (1987) A human single copy DNA probe (DR47) detects a Taq I RFLP on chromosome 9 (D9S5). Nucleic Acids Res 15:6310

Raimondi E, Antonelli A, Driesel AJ, Pandolfo M. Regional localization by in situ hybridization of a human chromosome 9 marker tightly linked to the Friedreich's ataxia locus. Hum Genet (in press)

Richter A, Melancon S, Farrall M, Chamberlain S (1989) Friedreich's ataxia: confirmation of gene localization to chromosome 9 in the Quebec French Canadian population. HGM10. Cytogenet Cell Genet 51:1066

Romeo G, Menozzi P, Ferlini A, Fadda S, Di Donato S, Uziel G, Lucci B, et al (1983) Incidence of Friedreich ataxia in Italy estimated from consanguineous marriages. Am J Hum Genet 35:523–529

Smith CL, Cantor RC (1986) Pulsed field gel electrophoresis of large DNA molecules. Nature 319:701–702

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396