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Linkage Analyses of Five Chromosome 4 Markers Localizes the Facioscapulohumeral Muscular Dystrophy (FSHD) Gene to Distal 4q35

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Summary

The genetic locus for facioscapulohumeral muscular dystrophy (FSHD) has been mapped to chromosome 4. We have examined linkage to five chromosome 4q DNA markers in 22 multigenerational FSHD families. Multipoint linkage analyses of the segregation of four markers in the FSHD families and in 40 multigenerational mapping families from the Centre d'Etude du Polymorphisme Humaine enabled these loci and FSHD to be placed in the following order: cen–D4S171–factor XI–D4S163–D4S139–FSHD–qter. One interval, D4S171–FSHD, showed significant sex-specific differences in recombination. Homogeneity tests supported linkage of FSHD to these 4q DNA markers in all of the families we studied. The position of FSHD is consistent with that generated by other groups as members of an international FSHD consortium.

Introduction

Facioscapulohumeral muscular dystrophy (FSHD; MIM 158900) is a dominantly inherited neuromuscular disorder with characteristic early involvement of muscles of the face and of the shoulder girdle (Tyler and Stephens 1950). Recently, linkage of FSHD to a marker on chromosome 4, D4S171, was reported (Wijmenga et al. 1990). This linkage result has led to the search for and identification of at least three other linked markers. One such marker, D4S139, has been reported to be very close to the FSHD locus (Upadhyaya et al. 1990; Wijmenga et al. 1991).

We have genotyped five loci that map to distal 4q in 22 FSHD families. Four loci were also mapped on 40 multigenerational mapping families from the Centre d'Etude du Polymorphisme Humaine (CEPH). These data enabled us to construct a multipoint map of this region including the FSHD genetic locus. As part of our participation in an international consortium to find the genetic location of FSHD, we have contributed our data to the consortium for multipoint analyses. The pooled data set should result in better localization of the FSHD locus and should contribute toward the development of a diagnostic test. Multipoint analyses of the pooled data are contained in this issue of the *Journal* (Sarfarazi et al. 1992)

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Subjects and Methods

Families and Diagnoses

Families were recruited from many locations throughout the United States. Sixteen families were collected by S.J.J. (University of California, San Diego); four families were collected by C.H. (University of Rochester); and two families were collected by J.M. (Ohio State University). All families used in this study were composed of at least one parent and at least two affected offspring. Six families were related by

virtue of a common affected ancestor; the pedigrees have been described by Tyler and Stephens (1950). Our total FSHD family set was composed of 304 individuals: 133 affected, 66 normal relatives, 49 spouses, and 56 whose disease status remains uncertain. In the 22 FSHD families, there are 91 meioses (affecteds only) and 144 meioses (affecteds and normals). Pedigrees will be made available on request.

Our diagnostic criteria have been described elsewhere (Jacobsen et al. 1990). All individuals classified as affected or normal were diagnosed by one of us (P.S., C.H., R.G., or J.M.) or by a physician in a Muscular Dystrophy Association neuromuscular disease clinic who is experienced in diagnosis of muscular dystrophies. Individuals who were not examined by a physician were classified as "uncertain." Because of the variable onset of symptoms, any individual who was less than 20 years of age and was diagnosed as normal or who showed possible weak facial and/or shoulder girdle signs was classified as "uncertain." Individuals classified as uncertain were used in the linkage analyses but did not contribute toward the linkage to FSHD (see Linkage Analyses subsection). We observed two cases in which a clinically asymptomatic individual passed the mutant gene for FSHD to her offspring. These "normal" individuals were coded as affected, for the computer analysis.

In addition to the FSHD families, 40 multigenerational mapping families from CEPH were genotyped. These families without FSHD provide additional phase known meioses, allowing construction of a multipoint map of the DNA markers alone.

DNA Markers and Molecular Analyses

All markers used in this study have been described elsewhere. D4S139 (pH30) is a VNTR marker (Milner et al. 1989). *Hin*dIII, *Taq*I, and, in some families, *Msp*I were used to genotype these families. D4S163 (EFD139) is a VNTR marker (Altherr et al. 1991) and was obtained from Y. Nakamura (Cancer Research Institute, Tokyo). The use of multiple restriction enzymes for each locus served to confirm questionable genotyping results. D4S171 is a $(CA)_n$ repeat probe described by Weber and May (1990). D4S187 (SBU10) was isolated by walking from the Duchenne muscular dystrophy locus to chromosome 4 by using an X:4 translocation (Bodrug et al. 1991; R. G. Worton, personal communication); the X:4 translocation has been described by Bodrug et al. (1990). This locus detects a simple single-strand conformation polymorphism. PCR primers, amplification conditions, and gel electrophoresis were performed as described by Mills et al. (1992). D4S187 was genotyped only on the FSHD families. The factor XI locus detects an RFLP described by Butler and Parsons (1990). To score the factor XI locus, we modified the procedure as follows: By using the oligonucleotide primers described by Butler and Parsons (1990), human DNA was amplified in a 20-μl reaction containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris pH 8.3, 150 μM dNTP, 125 ng human DNA, and 0.1 μl α^{32}P dATP. The amplification protocol was 94°C for 1 min, 60°C for 0.5 min, 72°C for 6 min, for 30 cycles. Five microliters of the PCR product was digested, with *Hba*I, in a final volume of 12.5 μl, according to the manufacturer's recommended conditions, for at least 3 h at 37°C. The digested product was electrophoresed on an 8% polyacrylamide gel, and the gel was exposed to XAR film.

Human DNA was extracted from whole blood or from established lymphoblastoid cell lines. Restriction digests and Southern blot preparations and hybridizations were performed as described elsewhere (Jacobsen et al. 1990).

Linkage Analyses

Linkage analyses were performed using the computer program package CRI-MAP (P. Green, K. Falls, and S. Crooks, personal communication). FSHD was assumed to be inherited in an autosomal dominant fashion, and penetrance was assumed to be complete. Individuals whose diagnoses were uncertain were included in the analyses, and their FSHD status was listed as "unknown." Thus, they contributed information for the segregation of DNA markers but not of FSHD.

Linkage analyses were begun by constructing a map of the chomosome 4 DNA markers in the CEPH mapping families. Two informative markers, D4S163 and D4S139, were used as the nucleus of the map. The multipoint analysis proceeded by inserting the other markers in all possible positions. By using the stringent criterion of 1,000:1 odds, a preliminary multipoint map of these markers was constructed. As a test of the map, groups of two, three, or four adjacent markers were inverted with respect to the other markers. This map was tested for data errors according to the protocol described below. Edits were made, and the analyses were repeated.

With a multipoint map of the DNA markers that was used as a starting point, analyses were performed using the merged data from the FSHD families and the CEPH mapping families. The order of the markers

was set as determined by the previous multipoint analyses. The FSHD locus was inserted in all possible locations, and the $\log_{10}$ likelihoods were determined. All recombination fractions (θ) were converted to centimorgans by using the Kosambi mapping function (Ott 1985).

Data Checking

To determine whether there were any errors in our data, the following steps were taken: All films were scored independently by two individuals. DNAs from the FSHD families were checked for paternity, by hybridization to many highly informative markers (Jacobsen et al. 1990). Two markers were genotyped independently in the CEPH families by two different groups. D4S139 was genotyped at Collaborative Research, Inc. and by E.C.B.M.'s laboratory; and D4S163 was genotyped at Collaborative Research, Inc. and by J.M.'s laboratory and was submitted to CEPH as unpublished data. Several discrepancies were found when independently collected data were compared. When the source of the error was found, edits were made in the computer files. The unpublished data for D4S163 were not used in our analyses.

In addition, the CRI-MAP computer program has a data-checking option (CHROMPIC) that displays the grandparental origin of the alleles. Cases in which many siblings in one family are recombinant are likely instances of data errors. In these cases, the original films were reexamined, hybridizations were repeated with independent Southern blots, computer files were checked, and a data base of problem blots was screened. Gel-loading errors were tested by hybridizing blots to a Y-specific probe, pDP105 (Disteche et al. 1986). Several suspected data errors were found when PCR was used to genotype an individual. Amplification reactions for that individual and his or her parents (or siblings) were repeated. When errors were found, edits were made in the computer files, and the multipoint analyses were repeated.

Reexaminations and blood donations were requested from all individuals who were recombinant for the closest markers—i.e., D4S163 and/or D4S139—and the FSHD locus. Nine of the 12 individuals with crossovers have submitted to a reexamination, and 6 have had blood redrawn for confirmation of the molecular results. Examination of these individuals and repetition of the molecular analyses confirmed that these individuals were, in fact, recombinants.

Results

Linkage Analyses

Two-point analyses were performed on the data generated using our FSHD families and CEPH families (table 1). Significant lod scores ($Z \geq 3.0$) were obtained between the FSHD locus and all of the markers

Table 1

Two-Point Linkage of Chromosome 4 Markers and FSHD

	Z AT θ OF									
LOCUS AND GROUP	.01	.05	.10	.15	.20	.30	.40	θ_{max}	Z_{max}	χ^{2a}
D4S139:										
Sex-equal	13.61	17.19	16.97	15.65	13.81	9.26	4.25	.07	17.33	
Female	16.49	17.50	16.99	16.05	14.90	12.25	9.40	.05	17.5	.782
Male	14.62	17.19	17.48	17.10	16.40	14.51	12.35	.09	17.5	
D4S163:										
Sex-equal	6.42	12.17	12.97	12.30	10.97	7.33	3.24	.10	12.53	
Female	9.50	12.59	12.97	12.55	11.78	9.70	7.39	.09	12.56	.138
Male	9.91	12.57	12.98	12.73	12.18	10.61	8.83	.11	12.56	
D4S171:										
Sex-equal	−16.90	−2.46	2.33	4.16	4.72	3.94	1.98	.22	4.52	
Female	2.28	5.57	6.20	6.04	5.53	3.97	2.12	.12	5.82	5.98
Male	−12.98	−1.83	2.34	4.33	5.41	6.19	6.02	.32	5.82	
D4S187:										
Sex-equal	1.46	3.57	3.87	3.65	3.21	2.02	.82	.10	3.88	
Female	3.18	4.06	4.00	3.67	3.21	2.14	1.10	.07	4.10	1.01
Male	2.38	3.60	3.60	4.08	4.09	3.94	3.70	.18	4.10	

[a] $\chi^2 > 3.8$ (1 df, $P > .05$)

except factor XI. D4S171, D4S187, and factor XI were relatively uninformative in our FSHD families and thus gave poor Z values. Significant Z values were obtained for all pairs of DNA markers genotyped in the CEPH families (data not shown). Two-point analyses were performed on each family separately; all but one family (family 32) showed positive Z values, indicating linkage to the 4q markers. Family 32 is a small two-generation family with three affected sibs. One of the affected sibs has a crossover between D4S139 and FSHD. Since it was impossible to distinguish whether this family was linked to the 4q markers, a homogeneity test was performed using HOMOG and the M test. There was no evidence of heterogeneity.

For most two-point intervals, the male θ and the female θ were similar. However, there was a large difference in the θ's involving D4S171. In particular, a sex-specific Z_{max} of 5.82 was seen between D4S171 and FSHD, at a θ of .12 in females and .32 in males (table 1). To determine whether any of the sex-specific differences in the two-point Z values were significant, a χ^2 analysis was performed (Ott 1985). In the interval D4S171–FSHD, the χ^2 values were significant (table 1).

The two-point analyses indicate linkage but do not determine the order of the markers. Therefore, we used multipoint-analysis methods as described above. Initial multipoint analyses with the DNA markers could not place D4S187 in a defined position. The inability to place D4S187 is not due to lack of linkage; two-point Z values for D4S187 and three other markers were significant. For example, the two-point Z_{max} for D4S139 and D4S187 was 8.36 (sex-equal) at θ of .04. The inability to order this marker with significant odds, is likely due both to its close proximity to other markers and to a small number of jointly informative meioses in our FSHD families. The availability of CEPH genotypic data for D4S187 should lead to its inclusion in the map (see Mills et al. 1992). Thus, D4S187 was not included in subsequent multipoint analyses.

The best multipoint order obtained was D4S171–factor XI–D4S163–D4S139. As a test of the analyses, groups of two, three, and four adjacent loci were inverted, and their log likelihoods were calculated. All inversions supported the original placement of markers, with odds greater than 1,000:1 (data not shown).

To place the FSHD locus within this genetic map, the FSHD locus was inserted into all possible intervals, and Z values were calculated (table 2). The strongest support is for an order in which the FSHD locus is distal to the other markers. In addition, the FSHD locus could be excluded from two intervals: D4S163–D4S139 and D4S171–factor XI. Finally, with use of the best order, a multipoint map was constructed (figure 1). The best map spans 21.4 cM sex-equal, 29.2 cM in males, and 14.1 cM in females. The location of the FSHD locus is distal to all of the markers used in this study. The closest marker is D4S139, which is 8.6 cM (sex-equal) from the FSHD locus (fig. 1).

Table 2

Support for Location of FSHD Locus

Order of DNA Markers	Z
D4S171–Factor XI–D4S163–D4S139–**FSHD**	**17.28**
D4S171–Factor XI–**FSHD**–D4S163–D4S139	6.84
FSHD–D4S171–Factor XI–D4S163–D4S139	6.75
D4S171–Factor XI–D4S163–**FSHD**–D4S139	−4.22
D4S171–**FSHD**–Factor XI–D4S163–D4S139	−7.25

NOTE.—The FSHD locus was inserted in all possible intervals in the best-order map, and the $\log_{10}$ likelihoods were determined. The Z was determined by subtracting the Log_{10} likelihood of the FSHD locus unlinked.

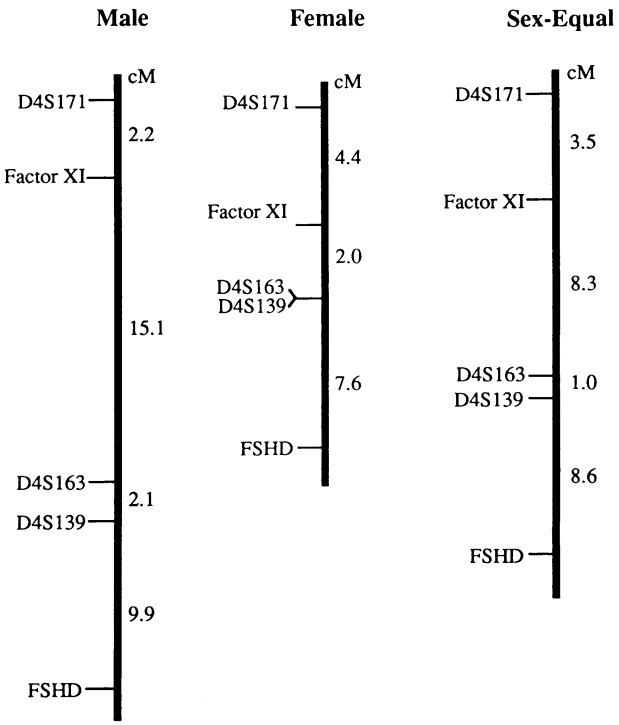

Figure 1 Multipoint map of distal 4q with sex-equal and sex-specific distances shown. θ's were converted to centimorgans by using the Kosambi mapping function (Ott 1985).

Recombination Events in FSHD Families

All of the crossovers detected in the FSHD families are shown in table 3, using the most likely order of the DNA markers. One double recombinant (out of 144 potentially informative meioses) is observed in our families, with one crossover between D4S171 and D4S163 and with the second crossover between D4S163 and D4S139 (individual 101-412). Twelve crossovers could be detected between the FSHD locus and the closely linked markers D4S163 and D4S139. Five of the recombinants are in individuals diagnosed as normal, and seven recombinants are in individuals diagnosed as affected.

Discussion

We have shown that the genetic locus for FSHD is linked to markers on chromosome 4. Our linkage results are in agreement with those of Upadhyaya et al. (1990) and Wijmenga et al. (1991), both of whom report linkage to D4S139. However, there are more recombinants between D4S139 and the FSHD locus in the families we studied, suggesting that the FSHD locus is at a greater distance from D4S139. In addition, the study by Wijmenga et al. (1991) could not determine on which side of the D4S139 locus the FSHD locus mapped. In the work described in the present paper, we have genotyped these markers on the CEPH mapping panel, which increases the likelihood of our multipoint map being correct. Similar multipoint results for these markers have been reported by Mills et al. (1992). In addition, the accompanying papers from members of the FSHD consortium support linkage of FSHD to the distal markers (Gilbert et al. 1992; Mathews et al. 1992; Mills et al. 1992; Sarfarazi et al. 1992; Upadhyaya et al. 1992; Wijmenga et al. 1992).

We see one double recombinant between D4S171 and D4S139 in the FSHD families (table 3). If we assume no interference, the frequency of such a double crossover event should be the product of the θ's for the interval between D4S171–D4S163 and D4S163–D4S139, or .107 × .01 = .00107. The predicted frequency is lower than we would expect, given the number of potentially informative meioses in the families we studied. However, we have seen one double recombinant between factor XI and D4S139 in the CEPH families, and other laboratories have seen double recombinants in their FSHD families (Upadhyaya et al. 1992; Wijmenga et al. 1992). The double recom-

Table 3

Crossovers in FSHD Families

Crossover Interval and Individual	Disease Status[a]	CHROMPIC[b] Result (D4S171–F11–D4S184–D4S139–FSHD)
D4S171–F11:		
13-445	U	0 1 1 1 *
D4S171–D4S163:		
11-45	A	0 * 1 1 1
2-28	A	0 * 1 1 1
2-4	N	0 * 1 1 1
102-506	N	0 * 1 1 1
201-429	A	0 * 1 1 1
2-524	A	1 * 0 0 0
1-113	A	1 * 0 0 0
4-58	N	1 * 0 0 0
116-309	N	1 * 0 0 0
11-38	U	0 * 1 1 *
26-571	A	0 * 1 * 1
12-340	U	1 * 0 0 *
101-415	U	1 * 0 0 *
D4S171–D4S163–D4S139:		
101-412	N	1 * 0 1 *
D4S171–D4S139:		
16-352	A	0 * * 1 1
16-361	N	1 * * 0 0
33-539	U	1 * * 0 *
33-544	U	0 * * 1 *
D4S171–FSHD:		
201-307	A	0 * * * 1
22-575	N	0 * * * 1
F11–D4S163:		
1-117	U	* 0 1 * *
3-267	U	* 1 0 0 *
D4S163–D4S139:		
11-37	U	1 * 1 0 *
33-532	U	0 0 0 1 *
D4S163–FSHD:		
11-36	A	0 * 0 * 1
22-410[c]	A	1 1 1 * 0
33-542	N	1 1 1 * 0
11-408[d]	N	1 * 1 * 0
D4S139–FSHD:		
6-78[d]	A	* * 1 1 0
101-515[c]	A	* * 1 1 0
12-333[c]	A	0 * 0 0 1
13-314[d]	N	0 * 0 0 1
32-563	A	0 * 0 0 1
101-501[c]	N	0 * 0 0 1
12-331[c]	N	0 * 0 0 1
101-507[c]	A	1 * 1 1 0

[a] A = Affected; N = normal; and U = uncertain or unknown.

[b] Displays the markers on the chromosomes in their most probable phase, with consistent representation of grandmaternal and grandpaternal alleles. 0 = grandmaternal; 1 = grandpaternal; and * = uninformative.

[c] Recombinants confirmed by reexamination and resampling.

[d] Recombinants confirmed by reexamination.

binants could reflect the high recombination frequency found in distal chromosomal regions. Twelve crossovers could be detected between the FSHD locus and the closely linked markers D4S163 and D4S139 (table 3). Two such recombinants are shown in figure 2. If FSHD is the most distal locus, each of these crossovers is most easily explained by a single recombination event. Placement of FSHD in another interval would necessitate multiple crossover events on the recombinant chromosome.

Significant excess of male recombinants has been reported for a number of distal chromosomal regions that contain many DNA markers—regions such as 1q (Buetow et al. 1990), 4p (Buetow et al. 1991), 5 (Weiffenbach et al. 1991), 10q (Bowden et al. 1989), 11q (Julier et al. 1990), 14q (Nakamura et al. 1989), 16 (Keith et al. 1990), and 17p (Wright et al. 1990). We also report excess of male recombinants in our map. The χ^2 analyses of the D4S171–FSHD interval shows significant excess of male recombination; there were no significant sex-specific differences in recombination in the intervals D4S139–FSHD and D4S163–FSHD (table 1). A closer examination of this region showed that the sex-specific differences in θ's mapped between factor XI and more distal markers (Weiffenbach et al., in press).

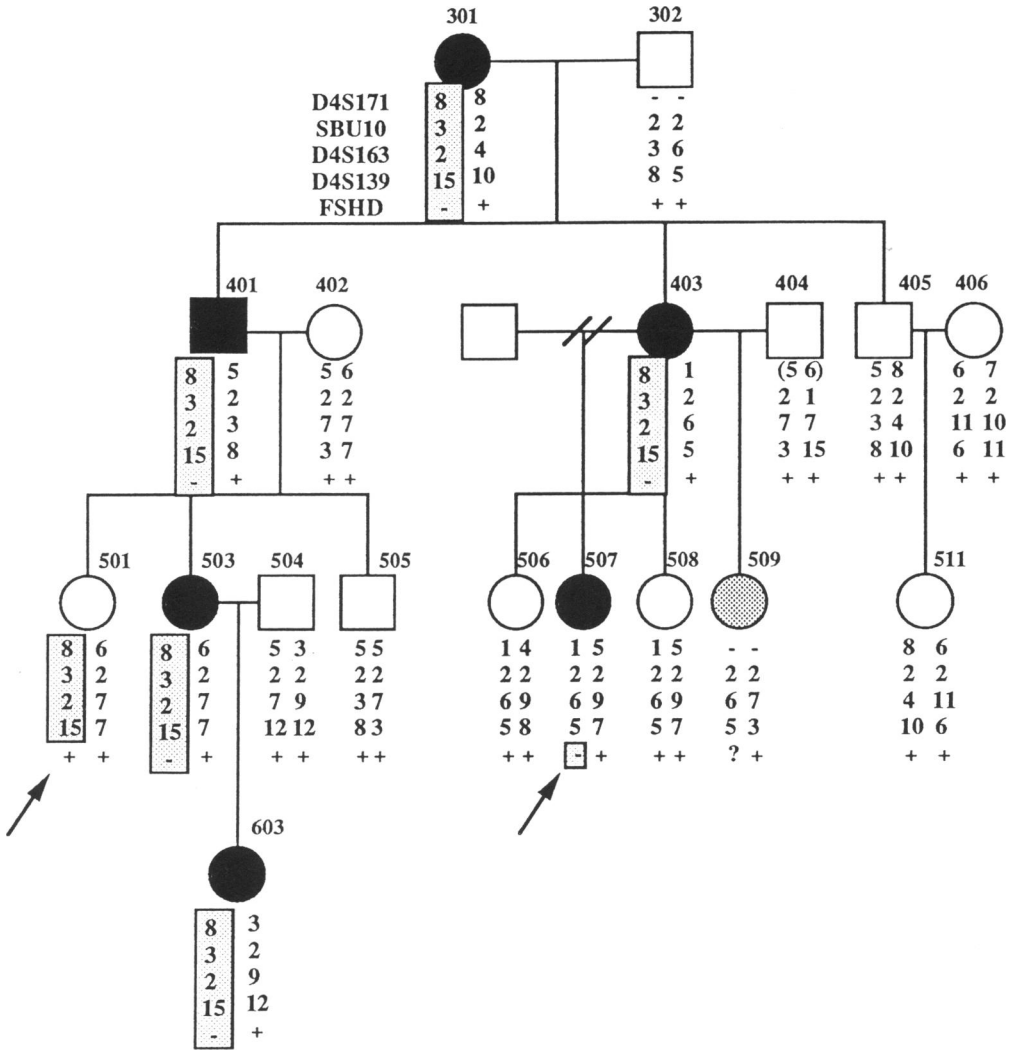

Figure 2 Family 101. Half of family 101 is shown with two recombinants, indicated by arrows. Individual identification numbers are above, and haplotypes for the 4q markers are displayed below the circles and squares. ● = Affected female; ■ = affected male; ○ = unaffected female; □ = unaffected male; and ◍ = status unknown.

FSHD is a progressive disease with varying age at onset. One interesting feature is the wide range of severity, even among members of the same family. This finding has been well documented in a large kindred from Utah, first described by Tyler and Stephens (1950). Many of the descendants of this pedigree are in our study. The variability of symptoms could interfere in studies such as this one if many participants who show such mild symptoms are classified as unaffected. The extent of misclassification would be apparent if a diagnosed normal individual passes the mutant gene to his or her offspring. Penetrance has been estimated to be 95% by the age of 20 years (Lunt et al. 1989). Our family set contains two obligately affected individuals who do not have clinically evident FSHD. One female is a member of the Utah kindred and has a son with typical FSHD muscle weaknesses. The mother, who was recently examined by one of us (P.S.), displays a slight transverse smile and a raised shoulder, symptoms that do not meet our criteria for affected status. The other obligate carrier is a mother in her 60s who has been examined twice (by J.M.) and has no features of FSHD. Her son, however, displays typical symptoms of FSHD shoulder and facial weaknesses. We do not believe that nonpenetrance is a particular problem in the family set we studied; we see approximately equal numbers of affected and normal recombinant individuals.

Ideally, results generated from research studies such as this one can be applied to diagnostic tests for interested FSHD families. Although this paper describes linkage to two highly informative VNTR markers, we feel that it is premature to use these markers in a diagnostic test, since the recombination rate is relatively high, even for the closest marker. As a number of research groups identify additional markers in the region, flanking markers will be isolated that will constitute a reliable diagnostic test. These new flanking markers will also be instrumental in defining the limits of the FSHD gene, for the ultimate goal of cloning this gene.

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