Exclusion mapping of chromosomal regions which cross hybridise to FSHD1A associated markers in FSHD1B

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Abstract

Facioscapulohumeral muscular dystrophy (FSHD) is a genetically heterogeneous, autosomal dominant primary disease of muscle. The predominant form of FSHD, which has been designated FSHD1A, has been localised to the 4q34 region of human chromosome 4. The disease locus (loci) for the remaining FSHD families, which are not linked to chromosome 4 and have been designated FSHD1B, has not yet been identified.

The D4F104S1 marker which detects copies of a 3.2 kb tandem repeat (D4Z4) which contains several types of repetitive sequences, including Hox gene-like elements, has been shown to be closely linked to the chromosome 4 FSHD disease locus. The loss of an integral number of the 3.2 kb tandem repeats has been associated with FSHD1A. When hybridised to chromosomal spreads these sequences cross hybridise with heterochromatin on acrocentric chromosomes and specific areas of human chromosomes 1, 3, and 10. Potentially these specific regions of cross hybridisation may be linked to FSHD1B. To examine this possibility we have carried out linkage studies in our largest FSHD1B family. In this paper we exclude these areas of specific cross hybridisation as disease loci for FSHD1B.

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Facioscapulohumeral muscular dystrophy (FSHD) is a slowly progressive primary disease of muscle that is inherited as an autosomal dominant disorder, although isolated and autosomal recessive cases have been described.¹ The incidence has been variously estimated at 1 to 10 cases per 100 000.² There is considerable variability in the age of onset and severity, but the disease usually manifests itself in the second to third decade of life.^{3,4} Early symptoms usually involve facial or shoulder girdle weakness. FSHD is progressive in the majority of patients spreading to the abdominal, foot, arm, and pelvic girdle muscles.⁴ In addition to muscle involvement, retinal vasculopathy and high frequency hearing loss is observed in some FSHD patients.^{5,6}

Linkage of FSHD1A was originally established to the 4q35-qter region.^{7,8} Subsequently, the isolation of the p13E-11 probe

(D4F104S1), which was developed from a cosmid isolated using a Hox probe, and which detects de novo rearrangements on sporadic and familial FSHD blots, led to further fine mapping of the chromosome 4 FSHD region.^{9,10} Specifically, in 10 Dutch families an *EcoRI* fragment shorter than normally observed in controls was found to co-segregate with FSHD1A. This has led to the suggestion that a defect in a homeobox gene associated with muscle development may be the underlying defect in chromosome 4q linked FSHD.¹⁰

The FSHD associated rearrangements detected by the p13E-11 probe were subsequently shown to be associated with the loss of integral numbers of copies of a 3.2 kb tandem repeat (D4Z4),^{11,12} although a number of crossovers have been observed.¹³ Each monomer of the 3.2 kb repeat has been shown by sequencing to contain two homeoboxes and additional repetitive sequences such as LSau.¹² In addition to the 4q35 region the repeat also cross hybridised to the heterochromatic pericentromeric and satellite bands on all of the acrocentric chromosomes and to specific areas on chromosome 1q12, 3p12, 10cen, and 10qter.¹²

Subsequent linkage studies have excluded several large FSHD families (FSHD1B) from the 4q FSHD1A region and shown FSHD to be a heterogeneous disorder.^{14,15} Potential candidate regions for the localisation of the FSHD1B families are those chromosomal locations which cross hybridise to the 3.2 kb repeat closely associated with the chromosome 4 FSHD gene. In this report we investigate the possibility of linkage between FSHD1B and the repeat cross hybridising regions of human chromosomes 1, 3, and 10.

Methods

FAMILY STUDIES

Duke FSHD family 689 was ascertained for study through the Muscular Dystrophy Association clinic at the Duke University Medical Center. Family 689 has been previously excluded from linkage to the 4q35 region.¹⁴ The clinical evaluation of this family has also been previously described.¹⁴ Briefly, the criteria of Lunt *et al*¹⁶ were used in assigning clinical status and all family members used in this study were examined by a neurologist before assignment of affected status. Affected members of Duke family 689 met the clinical criteria for FSHD¹⁶

including facial weakness, clavicular flattening, scapular winging, proximal muscle weakness, and myopathic changes on muscle biopsy without inflammatory or mitochondrial pathology. No obvious clinical differences between FSHD1A families and family 689 were observed. Blood was obtained at the time of clinical examination for DNA extraction and creatine kinase analysis from a total of 89 family members. For this study, a subset of the family including DNA samples on 32 affected subjects, 13 asymptomatic at risk subjects, and eight unrelated spouses was used.

DNA ISOLATION, MARKERS, AND LINKAGE ANALYSIS

DNA isolation and marker analysis were as previously described.¹⁴ Three markers were chosen for analysis in each of the four chromosomal areas that showed cross hybridisation to the 3.3 kb homeobox repeat sequence for a total of 12 markers. The 12 markers chosen were AMY2B, D1S185, and SPTA1 for chromosome 1q12¹⁷; D3S1217, D3S1284, and D3S1215 for chromosome 3p12¹⁸; D10S111, D10S174, and D10S196 for chromosome 10cen¹⁸; and D10S187, D10S216, and D10S212 for chromosome 10q26.^{18,19} Details regarding these markers, their heterozygosities, and their cytogenetic localisations are shown in table 1. These markers are all highly informative markers which flank the regions of homeobox cross hybridisation. The three chromosome 1 markers span a region of 44 cM on 1q12; the three chromosome 3 markers 35.9 cM on 3p12; and the chromosome 10 markers 40.8 cM on 10q26 and 22.5 cM on 10cen.

FSHD1B was analysed as an autosomal dominant disorder with age dependent penetrance. At risk subjects (asymptomatic offspring

of FSHD affected patients) included in the analysis were assigned probabilities of carrying the FSHD gene on the basis of their age at the time of examination. The risk calculations were based on the data of Lunt *et al*¹⁶ with a maximum penetrance of 93% at the age of 20 and above. In addition, in a conservative approach, an affecteds only (low penetrance) analysis was performed which used genotypic information on all family members, but included phenotypic data with respect to disease status only on known affected patients. The frequency of the FSHD gene was set at 0.0001 for all linkage analyses, and the marker allele frequencies were computed from the family data using the USERM13 version of the Mendel analysis program.²⁰

Simulation analysis of family 689 was performed to assess the power of this family to detect linkage. Analyses were carried out with the SIMLINK computer program^{21,22} using both complete family information, with risk classes outlined above, and using affecteds only.

Two point linkage analysis was used in subprogram MLINK of the LINKAGE program package (version 5.15). The multipoint analyses were performed using the FASTLINK version^{23,24} of the LINKMAP subprogram of LINKAGE.²⁵ Since the markers used are multi-allele marker systems, the markers were recoded to three four allele systems for computational feasibility²⁶ in the multipoint analysis. To ensure that the recoding of alleles did not alter the interpretation of results, the two point lod scores calculated using the full set of alleles were compared to those generated from the recoded alleles and only minor variations were seen between the two sets of results. Multipoint location scores were calculated as the log₁₀ likelihood difference of FSHD1B placed at a relative position on a fixed map of loci, versus the unlinked state. Map distances were converted to recombination fractions allowing for a Kosambi level of interference.

Visual inspection of the genetic data for each of the four candidate regions was performed using the two most widely spaced markers flanking the region in affected family members only. A region was considered to be excluded when at least two affected family members had entirely different haplotypes inherited from their affected parent.

Results

Simulation studies using both an age adjusted and a low penetrance analysis verified that Duke 689 is capable of yielding significant lod scores ($Z > 3.00$) with 92% probability for

Table 1 Markers and genetic maps used for investigation of candidate regions for FSHD family 689. Note that markers marked* were used for cytogenetic localisation only and were not genotyped in the pedigree

Marker	Heterozygosity	Genetic distance (cM)	Cytogenetic localisation
<i>Region 1q12</i>			
AMY2B	0.64	25	1p21
D1S185	0.49		
SPTA1	0.50	19	1q21
<i>Region 3p12</i>			
D3S1217	0.83	9.2	3p14.2-14.1
D3S1284	0.74		
D3S1215	0.78	26.7	3p13-p12
<i>Region 10cen</i>			
D10S111/ D10S174	0.62 0.75	18.3	10pter-p11.2
RBP3*			
D10S196	0.79	4.2	10q11.2
<i>Region 10q26</i>			
D10S187	0.82	14.6	10q26.1
D10S12*			
D10S216	0.60	4.7	
D10S212	0.71	21.5	

Table 2 Results from simulation analysis for FSHD1B family 689

θ	Age adjusted		Affecteds only	
	Mean lod	Max lod	Mean lod	Max lod
0.00	6.15	10.2	5.26	8.34
0.05	4.39	9.79	3.72	7.98
0.10	3.13	8.30	2.63	7.91
0.15	2.22	9.12	1.86	7.13
0.20	1.45	7.15	1.26	5.74

Haplotype analysis of representative portions of FSHD1B family 689 for each of the four chromosomal regions examined. Markers for chromosome 1 are *AMY2B*, *D1S185*, and *SPTA1*; for chromosome 3 *D3S1217*, *D3S1284*, and *D3S1215*; for chromosome 10cen *D10S111*/*D10S174*, *RBP3*, and *D10S196*; and for chromosome 10q *D10S187*, *D10S216*, and *D10S212*, top to bottom respectively. Recombinations between the FSHD1B and the chromosomal markers are clearly observed.

markers 5 cM from the disease gene. The results can be seen in table 2. Table 3 shows the two point lod scores in FSHD1B family 689 for both the age adjusted and affecteds only analysis for each of the three markers in the four chromosomal regions which cross hybridised with the 3.2 kb repeats. The majority of the two point lod scores were negative for all members tested for both the age adjusted and affecteds only analysis. There was no evidence for significant linkage to any of the regions. The highest lod score realised was 0.85 for *D10S216*; the flanking markers to *D10S216* (*D10S187* and *D10S212*) were, however, neg-

ative. Multipoint linkage analysis using both the age adjusted and low penetrance analyses excluded the regions between the most distant markers flanking the candidate region (that is, lod scores < -2.0) for the 1q, 3p, and 10cen regions; the 10q multipoint lod scores were negative throughout the candidate interval, but did not reach the standard exclusion criteria of lod scores < -2.0 . Similarly, the low penetrance analysis allowed exclusion of the 1q and 10cen areas, while the 3p and 10q candidate intervals had negative or slightly positive (peak lod score = 0.33 in the 3p region) lod scores within the interval. In addition, each of the four

Table 3 Two point lod scores for markers in homeobox regions for FSHD1B family 689

Locus	Recombination fraction ($\theta_M = \theta_F$)						
	0.00	0.05	0.10	0.15	0.20	0.30	0.40
(A) Age adjusted analysis							
1q12:							
AMY2B	-∞	-3.32	-2.04	-1.40	-1.04	-0.62	-0.31
D1S185	-∞	-8.33	-4.76	-2.86	-1.69	-0.47	-0.10
SPTA1	-∞	-8.07	-5.44	-3.95	-2.95	-1.59	-0.66
3p12:							
D3S1217	-∞	-6.47	-3.85	-2.33	-1.35	-0.26	0.12
D3S1284	-∞	-2.46	-1.25	-0.60	-0.23	-0.10	0.13
D3S1215	-∞	-4.82	-2.58	-1.44	-0.76	-0.14	-0.01
10cen:							
D10S111	-∞	-3.02	-1.40	-0.63	-0.19	-0.21	0.24
D10S174	-∞	-5.97	-3.05	-1.54	-0.64	-0.19	0.30
D10S196	-∞	-6.51	-3.94	-2.57	-1.71	-0.72	0.21
10q26:							
D10S187	-∞	-5.09	-2.71	-1.50	-0.80	-0.16	-0.04
D10S216	-∞	0.72	0.85	0.84	0.76	0.52	0.25
D10S212	-∞	-3.01	-1.34	-0.50	-0.03	0.34	0.29
(B) Affecteds only							
1q12:							
AMY2B	-∞	-3.50	-2.20	-1.53	-1.12	-0.61	-0.28
D1S185	-∞	-6.58	-3.48	-1.90	-0.97	-0.11	0.04
SPTA1	-∞	-6.61	-4.28	-3.04	-2.23	-1.18	-0.49
3p12:							
D3S1217	-∞	-5.07	-2.91	-1.69	-0.93	-0.10	0.15
D3S1284	-∞	-1.69	-0.65	-0.15	0.11	0.25	0.17
D3S1215	-∞	-4.11	-2.08	-1.07	-0.51	-0.02	0.04
10cen:							
D10S111	-∞	-2.87	-1.35	-0.63	-0.23	-0.13	0.19
D10S174	-∞	-3.99	-1.56	-0.40	0.23	0.63	0.42
D10S196	-∞	-6.12	-3.73	-2.47	-1.69	-0.77	-0.27
10q26:							
D10S187	-∞	-4.58	-2.35	-1.25	-0.63	-0.10	-0.03
D10S216	-∞	-0.00	0.21	0.29	0.31	0.27	0.17
D10S212	-∞	-3.49	-1.70	-0.78	-0.25	0.20	0.22

candidate regions was excluded by haplotype analysis in affected family members (figure).

Discussion

FSHD has recently been shown to be a heterogeneous disorder. This finding has important implications for both genetic counselling and the isolation of the 4q35 FSHD gene. Many FSHD families are often too small to allow definitive determination of whether they are chromosome 4 linked. While the development of the p13-11 probe has been an important diagnostic tool, the correlation between the loss of a number of 3.2 kb repeats and FSHD1A is not absolute.¹³ In addition, interpretation of the results obtained from the use of p13-11 is often quite difficult. The linkage and identification of the second FSHD locus and potentially additional loci which may cause the disease is of great importance for FSHD diagnosis, treatment, and mutation analysis.

One obvious location for a second locus would be those distinct areas of chromosome 1q, 3p, 10cen, and 10q which cross hybridise with the homeobox repeat. In this study we have examined four of these chromosomal areas in a large, confirmed non-chromosome 4 linked FSHD family. Using both linkage analysis and

haplotype analysis these four regions have been excluded as the FSHD locus in family 689 in their entirety.

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