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Linkage Studies in Facioscapulohumeral Muscular Dystrophy (FSHD)

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Summary

Facioscapulohumeral muscular dystrophy (FSHD) has been localized to the 4q35-qter region of chromosome 4. Linkage analyses of two polymorphic markers from the region, D4S139 and D4S163, have been carried out using four large multigenerational FSHD families. The results indicate that both markers are closely linked to FSHD, with D4S139 being the closest proximal marker to FSHD.

Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is a slowly progressive primary disorder of muscle. It is usually inherited as an autosomal dominant disorder, although isolated and/or autosomal recessive cases have been described (Moser et al. 1966). The incidence is reported to be 3–10 cases/100,000 (Morton and Chung 1960). There is often marked variability in both age at onset and clinical severity (Walton and Gardner-Medwin 1981). Mild symptoms may exist for years before they become bothersome enough for patients to seek medical attention. Typically the disease manifests in the second or third decade, but earlier onset is not uncommon (Dubowitz 1985). The molecular defect underlying FSHD is unknown.

FSHD has recently been localized to the long arm of chromosome 4, by using the microsatellite repeat D4S171 (MFD22) (Wijmenga et al. 1990). Subsequent linkage studies with D4S139 (pH30) sublocalized FSHD to the 4q35-qter region (Upadhyaya et al. 1990, 1991; Wijmenga et al. 1991). In the present study, the two previously reported FSHD-linked markers, D4S139 and D4S163 (EFD139.1) (Neuweiler et al. 1990), were analyzed in four FSHD families to confirm the linkage in these families and to map FSHD relative to these marker loci.

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Material and Methods

Family Studies

FSHD families, with the exception of family 953, were ascertained for study through the Muscular Dystrophy Association Clinic at Duke University Medical Center. Family 953 was ascertained at the Salpetrière Hospital in Paris. All participating family members were examined by a neurologist before assignment of affection status. The criteria of Lunt et al. (1988) were used in determining clinical status. All four families demonstrated unequivocal signs of FSHD: facial weakness, shoulder-girdle weakness/wasting, and scapular winging either at rest or with resistance. Over 100 individuals (51 affected with FSHD) in four large multigenerational FSHD families were included in the study. Blood was obtained at the time of examination, for creatine kinase analysis as well as for DNA extraction. There was significant variability in the degree of severity within families and even within sibships.

DNA Isolation and DNA Markers

Family DNA was isolated as described elsewhere (Pericak-Vance et al. 1988). The VNTR markers pH30 and EFD139.1 were used to detect polymorphisms on Southern blotting, by using the restriction enzymes *Msp*I and *Hin*dIII, respectively. DNA was

Table 1

Linkage Analyses of Chromosome 4 Markers D4S139 and D4S163 Versus the FSHD Locus and Marker-to-Marker Z Values

Locus vs. Locus	Probe	$Z(\hat{\theta})$	$\hat{\theta}$	95% Confidence Intervals
FSHD vs. D4S139	pH30	7.57	.02	.01–.11
FSHD vs. D4S163	EFD139.1	6.80	.04	.01–.14
D4S139 vs. D4S163	. . .	8.90	.04	.01–.12

NOTE.—Analyses included four FSHD families with 51 affecteds.

digested (2 ×) using the appropriate enzyme under conditions recommended by the manufacturer and was electrophoresed on a 0.7% agarose gel. DNA was blotted onto Hybond N+ (Amersham) and hybridized as described elsewhere (Pericak-Vance et al. 1988). pH30 and EFD139.1 were labeled using the Multiprime DNA system (Amersham).

Linkage Analysis

Two-point analyses were performed by the computer program LIPED (Ott 1974). The multipoint analyses were performed using the LINKMAP subprogram of the computer package LINKAGE, version 4.9 (Lathrop et al. 1984). Multipoint lod score (Z) values were calculated as $\log_{10}[L(x)/L(x = .5)]$, where x is the distance of FSHD relative to a fixed point on a given map of the markers. A distance of 3 cM was assumed between the markers D4S139 and D4S163 in the multipoint analysis, as determined by Sarfarazi et al. (1992). Since both D4S139 and D4S163 are VNTR markers with many alleles, the markers were downcoded to two four-allele systems in preparation for the analyses. This was necessary since the analysis was too computer-time intensive with these large multigenerational families. FSHD was analyzed as an autosomal dominant disorder with age-dependent penetrance. At-risk individuals included in the analysis were assigned probabilities of carrying the FSHD gene, on the basis of their age at examination. The risk calculations were generated from a normal distribution based on the data of Lunt et al. (1988), with maximum penetrance of 93% at age 20 years and above. All undiagnosed family members were coded as unknown with respect to disease status.

Results and Discussion

Results of the two-point analyses between FSHD and the marker loci are given in table 1, together with their approximate 95% confidence limits (Ott 1991). D4S139 gave the highest Z value, with $Z(\hat{\theta}) = 7.57$ at recombination fraction $(\hat{\theta}) = .02$. D4S163 gave a somewhat lower score, with $Z(\hat{\theta}) = 6.80$ at $\hat{\theta} = .04$. When FSHD was moved through the fixed map of cen–D4S163–D4S139–qter, a peak multipoint Z value of 10.30 was attained when FSHD was 2 cM distal to D4D139 (fig. 1). This placement was favored with odds of 15:1 over the next most likely order, which occurred when FSHD was approximately 2 cM proximal to D4S163.

A key affected recombinant individual was identified in Duke family 650 and is depicted in figure 2. Individual 0105 is crossing-over for both D4S139 and D4S163. The clinical findings in this patient include weakness of neck flexors and of the proximal and distal upper and lower extremities, clavicular flattening, and scapular winging at rest, which was accentuated with resistance. Diagnosis of FSHD was unequivocal. Individual 0105 is the only affected-affected crossover in our four families with D4S139. Analyses indicate

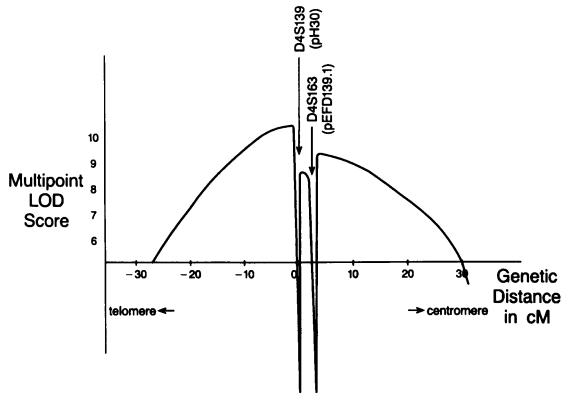

Figure 1 Multipoint linkage results for FSHD and the markers D4S139 and D4S163. The multipoint Z value was calculated as the $\log_{10}[L(x)/L(x = .5)]$.

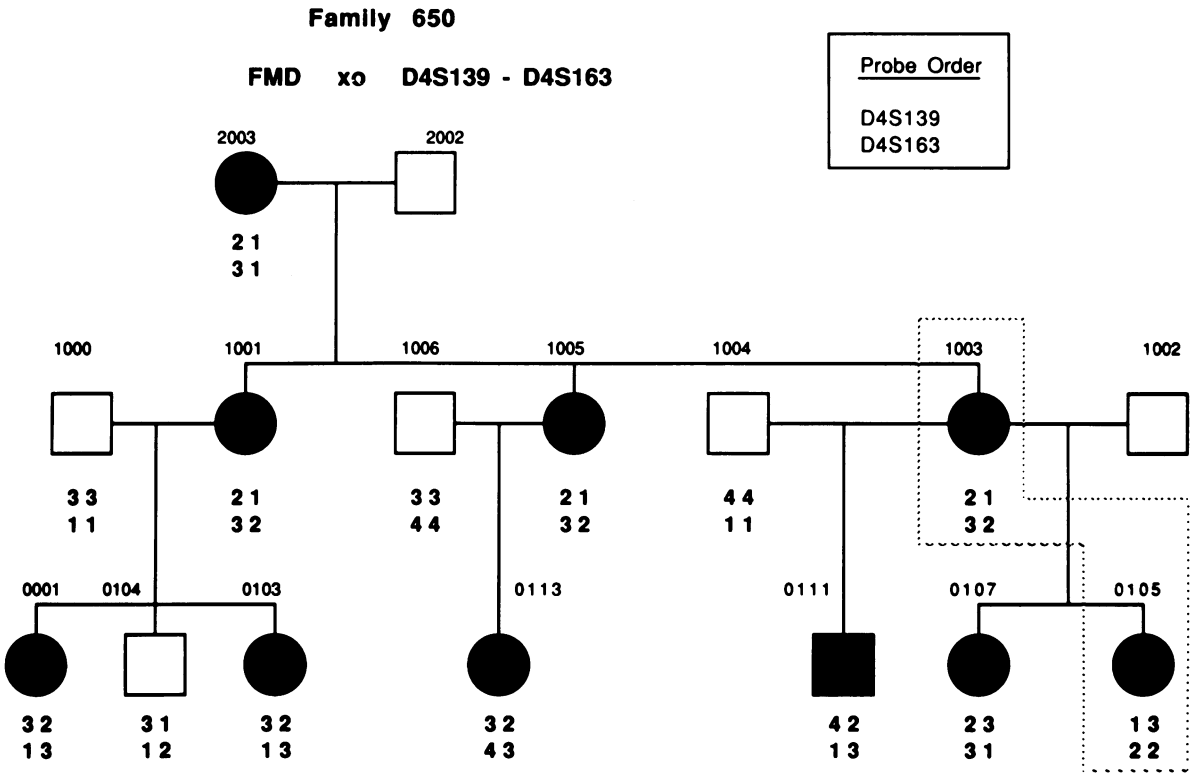

Figure 2 Crossover between FSHD and D4S139 and D4S163 in family 650. Upper boldface numbers are alleles assigned to D4S139, while lower boldface numbers represent D4S163 alleles. The critical crossover between individuals 1003 and 0105 is boxed.

that, at present, D4S139 is the closest known probe to the FSHD locus.

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