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ORIGINAL INVESTIGATION

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Chromosome 4q35 haplotypes and DNA rearrangements segregating in affected subjects of 19 Italian families with facioscapulohumeral musculatur dystrophy (FSHD)

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Abstract Four DNA markers on the distal long arm of chromosome 4 have been analyzed for their linkage to facioscapulohumeral muscular dystrophy locus (FSHD) in a series of 16 Italian families. We found that, in two families, the disease is not linked to the 4q35 markers, indicating the presence of genetic heterogeneity among Italian FSHD families. Linkage analysis in the remaining families supports the order cen-D4S171-D4S163-D4S139-D4S810-FSHD-qter, in agreement with the physical map from the literature. *Eco*RI digestion and hybridization with the distal marker p13E-11 (D4S810)[1] detected DNA rearrangements in the affected members of both sporadic and familial cases of FSHD, with family-specific fragments ranging in size between 15 kb and 28 kb. In three sporadic FSHD cases, the appearance of a new "small" fragment not present in either parent was clearly associated with the development of FSHD disease. However, in the familial cases analyzed, we observed two recombinations between all four 4q35 markers and the disease locus in apparently normal subjects, leaving open the possibility of nonpenetrance of the FSHD mutation.

[1] The locus D4S810 corresponding to probe p13E-11 has been recently renamed D4F104S1.

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Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder with a prevalence of 1:20000 (Padberg 1982; Lunt and Harper 1991), characterized by early involvement of muscles of the face and of the shoulder girdle. Other muscles are affected by the disease in a specific order, abdominal muscles first, followed by foot extensor and upper arm muscles, and finally by pelvic girdle and lower arm muscles. Patients with a negative family history have been frequently described and probably arise because of new mutations. The disease shows considerable variation in age of onset and clinical severity, both between and within families, ranging from almost asymptomatic forms to more severe wheel-chair-bound forms. The penetrance is estimated to be 70% by the age of 15 years and 95% after 20 years (Lunt et al. 1988).

The gene responsible for FSHD has been localized to the subtelomeric portion of chromosome 4 long arm (q35-qter), 4 cM distal to the linkage group D4S171-F11-D4S163-D4S139 (Sarfarazi et al. 1992; Upadhyaya et al. 1992; Wijmenga et al. 1992a; Weiffenbach et al. 1992; Gilbert et al. 1992; Mathews et al. 1992; Mills et al. 1992). Recently, a probe derived from cosmid 13 E, isolated in an independent search for a human homeobox gene, has been shown to detect polymorphic DNA fragments in normal subjects, "de novo" DNA rearrangements is smaller than 28 kb in sporadic cases, and similar "small" fragments in familial FSHD patients (Wijmenga et al. 1992b). These "small" fragments in the range 14 kb to 28 kb were proposed to be the site of DNA rearrangements causing the disease (Wijmenga et al. 1992b). However, a recent paper by Weiffenbach et al. (1993) suggests a more complicated picture, reporting six affected individuals who did not display a family-specific FSHD fragment and six unaffected who exhibited a "small" fragment. We have tested chromosome 4q35 markers (D4S171, D4S163, D4S139, D4S810) for linkage to the FSHD locus in a set of 16 Italian FSHD families and have searched for DNA rearrangements by probe p13E-11 in 16 FSHD families and three sporadic FSHD cases.

Materials and methods

Family studies

A total of 16 FSHD families and three sporadic cases were recruited through the collaboration of Neurology Departments in Rome and Milan. The 16 pedigrees included 111 individuals consisting of 55 affected and 56 unaffected persons. All family members were examined by a neurologist before assigning the affected status. Diagnostic criteria followed the guidelines proposed by the International Consortium (Padberg et al. 1991). In each kindred, at least one patient had a muscle biopsy and electromyography to confirm the diagnosis of FSHD.

DNA analysis

DNA was extracted from lymphocyte nuclei, digested with the appropriate enzyme, fractionated on 0.8% agarose gels, and transferred by Southern blotting to nitrocellulose or nylon filters (Di Rienzo et al. 1985). DNA probes were labeled with α-^{32}P by random oligonucleotide priming. Standard polymerase chain reactions (PCR) were carried out in 50 µl containing 200 ng genomic DNA, 150 ng of each oligodeoxynucleotide primer (Neuweiler et al. 1990), 6 ng CA strand primer 5'-labeled with α-^{32}P, 125 µ*M* of each dNTP, 50 m*M* KCl, 10 m*M* TRIS-HCl pH 8.3, 0.8 m*M* $MgCl_2$, and 1 U *Taq* polymerase (Perkin Elmer Cetus). Samples were overlaid with mineral oil and were processed through 27 temperature cycles of 1 min at 94°C (denaturation), 2 min at 55°C (annealing), and 1 min at 72°C (extension). The last elongation step was lengthened for 6 min. Samples of one tenth of the amplified DNAs were subjected to electrophoresis on standard denaturing polyacrylamide DNA sequencing gels. After being fixed and dried, the gels were exposed for 1 day.

Linkage analysis

The DNA probes detecting polymorphisms used in the linkage studies (D4S171, D4S163, D4S139, D4S810) were kindly provided by Dr. R. Frants from the Department of Human Genetics, Leiden University, The Netherlands (see Table 1). Two-point linkage analysis was performed by the computer package LINKAGE (Lathrop et al. 1984). FSHD was assumed to be inherited in an autosomal dominant fashion. Because of the age-dependent phenotype, five liability classes were used according to the following age intervals: less than 4 years, 5–9 years, 10–14 years, 15–19 years, more than 19 years of age. The probability of being affected for each liability class was derived from cross-sectional analysis of age-dependent segregation ratios in 143 at-risk individuals as reported by Lunt et al. (1988).

Multiallele systems were reduced according to the method reported by Ott (1978). Taking into account the large number of alleles observed in the sample of Italian families, the frequencies of private alleles were conservatively assumed to be 0.10 for the D4S139 and D4S163 loci, and 0.20 for D4S171.

Probe p13E-11 (D4S810) reveals a complex pattern of bands ranging from 10 kb to 40 kb on standard agarose gel electrophoresis, since it recognizes two polymorphic loci and a 10-kb Y-specific fragment (R. Frants, personal communication). In this case, a two-allele system was used, scoring for the presence or absence of a "small" (< 28 kb) non-Y fragment. In three families showing more than a single "small" fragment, the smallest was scored. A genetic homogeneity test among FSHD families was performed with the computer program HOMOG, version 2.80 (Ott 1983).

Results

Linkage studies with polymorphic 4q35 markers

The results of two-point linkage analysis between the FSHD locus and the 4q35 markers in 16 FSH families are shown in the upper part of Table 2. The Θ_{max} values for the different markers are much higher than those reported in a paper by the International Consortium (Sarfarazi et al. 1992). A possible explanation is that genetic heterogeneity is present in the Italian sample of FSHD families, leading to an overestimation of genetic distances. Evidence of genetic heterogeneity was found in two out of seven FSHD families from the United States (Gilbert et al. 1993). For

Table 1 Polymorphic chromosome 4q35 markers used for linkage analysis

Probe	Locus	Enzyme	Type	Alleles	References
Mfd22	D4A171	None[a]	(CA)n	143–163 bp	Weber and May (1990)
L1LA5	D4S163	*Pst*I	VNTR	5–18 kb	Neuweiler et al. (1990)
pH30	D4S139	*Taq*I	VNTR	4–20 kb	Milner et al. (1989)
p13E-11	D4S810	*Eco*RI		13–300 kb	Wijmenga et al. (1992b)

[a] Denaturing sequencing gels

Table 2 Two-point linkage analysis between the FSHD locus and 4q35 markers in 16 FSHD families

Locus	*Z* values at recombination fractions of											
	0.00	0.01	0.03	0.05	0.07	0.10	0.15	0.20	0.25	0.30	θ_{max}	Z_{max}
D4S171	$-\infty$	−5.318	−3.190	−1.967	−1.151	−0.350	0.3765	0.6882	0.7564	0.6690	0.24	0.76
D4S163	−4.039	2.8267	4.2243	4.7514	4.9766	5.0275	4.6954	4.0756	3.2898	2.4331	0.09	5.04
D4S139	−3.045	2.6185	4.0806	4.6802	4.9740	5.1155	4.8835	4.3189	3.6256	2.6766	0.10	5.11
D4S810	8.0569	8.2962	8.3092	8.1404	7.8892	7.4215	6.4911	5.4455	4.3312	3.1939	0.02	8.34
	Families FSH 03 and 12 excluded											
D4S171	$-\infty$	−1.466	−0.262	0.3131	0.6666	0.9822	1.1972	1.1917	1.0521	0.8269	0.171	1.21
D4S163	7.3216	7.3237	7.2303	7.0535	6.8187	6.3936	5.5535	4.6076	3.6028	2.5951	0.005	7.33
D4S139	8.6366	8.6195	8.4896	8.2732	8.0000	7.5135	6.5645	5.4999	4.4396	3.2126	0.001	8.64
D4S810	9.5204	9.3749	9.0554	8.7082	8.3375	7.7474	6.6924	5.5737	4.4141	3.2474	0.001	9.52

Table 3 Two-point linkage analysis between D4S139 and the FSHD locus

	0.00	0.01	0.03	0.05	0.07	0.10	0.15	0.20	0.30
Family 01	1.8898	1.9140	1.9301	1.9179	1.8867	1.8146	1.6469	1.4356	0.9190
Family 02	0.4876	0.4758	0.4521	0.4280	0.4037	0.3668	0.3045	0.2421	0.1244
Family 03	−3.985	−1.919	−1.355	−1.069	−0.876	−0.667	−0.444	−0.295	−0.116
Family 08	0.5359	0.5199	0.4880	0.4564	0.4250	0.3785	0.3035	0.2327	0.1112
Family 09	0.1487	0.1437	0.1338	0.1241	0.1146	0.1008	0.0792	0.0595	0.0274
Family 11	0.9027	0.8853	0.8498	0.8136	0.7767	0.7198	0.6209	0.5169	0.2977
Family 12	−7.697	−4.082	−3.054	−2.524	−2.150	−1.731	−1.237	−0.886	−0.420
Family 17	−0.440	−0.378	−0.283	−0.215	−0.163	−0.107	−0.049	−0.018	0.0035
Family 18	0.5808	0.5679	0.5418	0.5153	0.4882	0.4468	0.3759	0.3035	0.1612
Family 22	0.8838	0.8621	0.8183	0.7736	0.7282	0.6588	0.5404	0.4208	0.1976
Family 25	1.1828	1.1569	1.1042	1.0505	0.9957	0.9116	0.7667	0.6169	0.3179
Family 26	0.1472	0.2014	0.2783	0.3281	0.3601	0.3855	0.3873	0.3556	0.2302
Family 29	0.8818	0.8646	0.8296	0.7939	0.7574	0.7013	0.6039	0.5016	0.2872
Family 30	1.1557	1.1344	1.0915	1.0481	1.0042	0.9377	0.8250	0.7103	0.4766
Family 31	0.2798	0.2715	0.2551	0.2387	0.2225	0.1983	0.1593	0.1224	0.0587

this reason, we performed admixture analysis on two-point linkage data by using the HOMOG program (Ott 1983). Using the most informative D4S139 marker (Table 3), the null hypothesis of homogeneity in linkage was rejected with a χ^2 of 6.514 (1 *d.f.*, $P = 0.005$). The estimate of the proportion of linked families was 80% with $\Theta = 0.01$. The conditional probabilities of being linked for families FSH 12 and FSH 03 were low, viz., 0.0003 and 0.046, respectively. The lower part of Table 2 reports the results of two-point linkage analysis after exclusion of families FSH 12 and FSH 03.

DNA rearrangements detected by the distal marker p13E-11

Sporadic FSHD cases

Three FSHD patients with a negative family history (neither parent shows the clinical signs of the disease) were assumed to carry a new mutation and were tested with probe p13E-11. In all three FSHD patients, hybridization with p13E-11 detects *Eco*RI fragments that are not present in either parent, indicating that a "de novo" DNA rearrangement is associated with the development of FSHD disease. The rearranged *Eco*RI fragments have a different lenght in each patient, ranging in size between 15 kb and 23 kb, and appear to be smaller than those usually found in healthy individuals (> 28 kb).

Familial FSHD cases

Although the alleles larger than 28 kb are not clearly separated from each other, probe p13E-11 is useful in detecting a fragment, usually smaller than 28 kb, in the affected individuals of each FSHD family. The smallest non-Y *Eco*RI fragment in the range 14 kb to 28 kb segregating in each family is referred to as "small", whereas fragments that are larger than 28 kb and that are found in all individuals are referred to as "large" (see Fig. 1). Linkage analysis shows that "small" alleles segregate in tight linkage with D4S139 ($Z_{max} = 10.82$ at $\Theta = 0.016$). Thus, all of the "small" alleles in our series can be considered to be derived from the 4q35 region. In agreement with this observation, the "small" allele in families 12 and 03 does not recombine with D4S139, whereas it does not show linkage to FSHD. After removal of these two families, locus D4S810 shows a close overall linkage to FSHD (see Table 2, lower part).

It is interesting to note that we have observed a single recombination event between D4S810 and the other 4q35 markers. This identifies D4S810 as the closest marker to FSHD. The recombinant is a healthy subject currently aged 51 (family 26), who inherited no "small" allele at D4S810 but a 4q35 haplotype otherwise associated with FSHD in his affected sibs. Indeed, this family shows a peak lod score between D4S139 and FSHD at $\Theta = 0.15$ (Table 3).

The FSHD-associated "small" fragments could be assigned to different classes according to their size (Wijmenga et al. 1992b). We were able to detect "small" fragments in the range 14 kb to 22 kb that segregate with the disease in 57% of Italian families and fragments in the range 23 kb to 28 kb in 36% of families. One family (FSH 18) showed no "small" fragment.

4q35 Haplotypes, DNA rearrangements and clinical phenotypes in Italian FSHD pedigrees

In each family studied, it is possible to reconstruct the chromosome phase and identify a set of alleles at polymorphic markers associated either with the disease (affected haplotype) or with the normal phenotype (normal haplotype). However, two recombinations were observed between the entire set of 4q35 markers and the disease locus in two apparently normal subjects carrying the affected haplotypes associated with the "small" fragments.

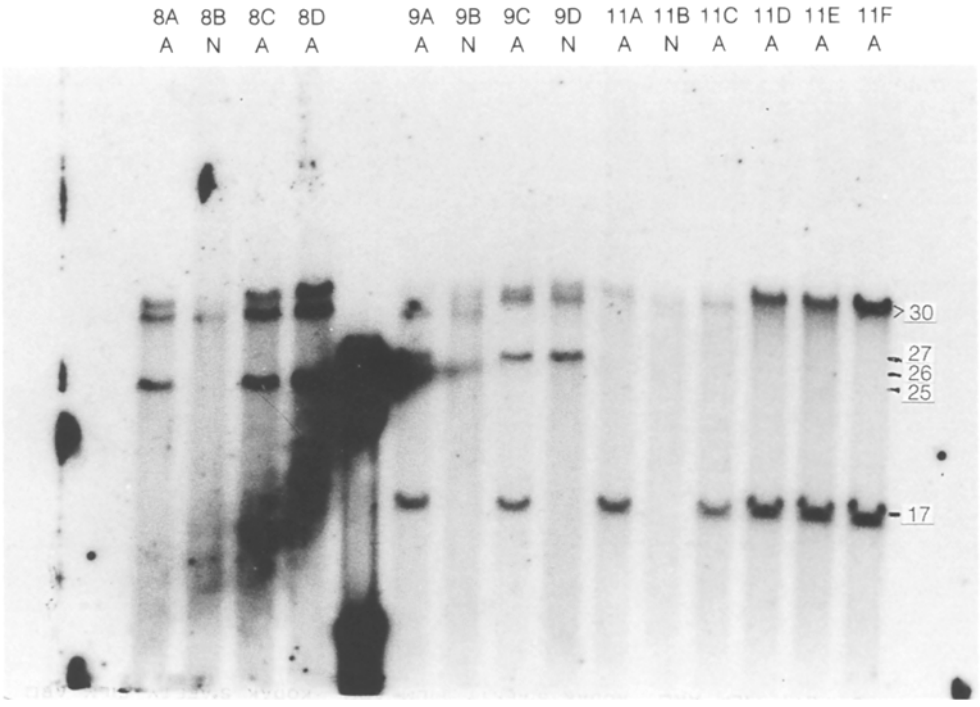

Fig. 1 Hybridization patterns of p13E-11 in three Italian FSHD families. In family FSH 08, a "small" fragment of 25 kb is present in affected *(A)* individuals *(lanes 8A, 8C, 8D)*, whereas it is absent in *lane 8B* (*N* normal). In family FSH 11, a "small" fragment of 17 kb is present in the affected *(A)* subjects *(lanes 11A, 11C, 11D, 11E, 11F)*, but is absent in *lane 11B* (*N* normal). In family FSH 9, a fragment of 17 kb is present in the affected *(A)* subjects *(lanes 9A, 9C)*, but is absent in normals *(N) (lanes 9B, 9D)*. In these normal individuals, fragments of 26 kb and 27 kb are detected; these appear to be polymorphic fragments not related to the disease

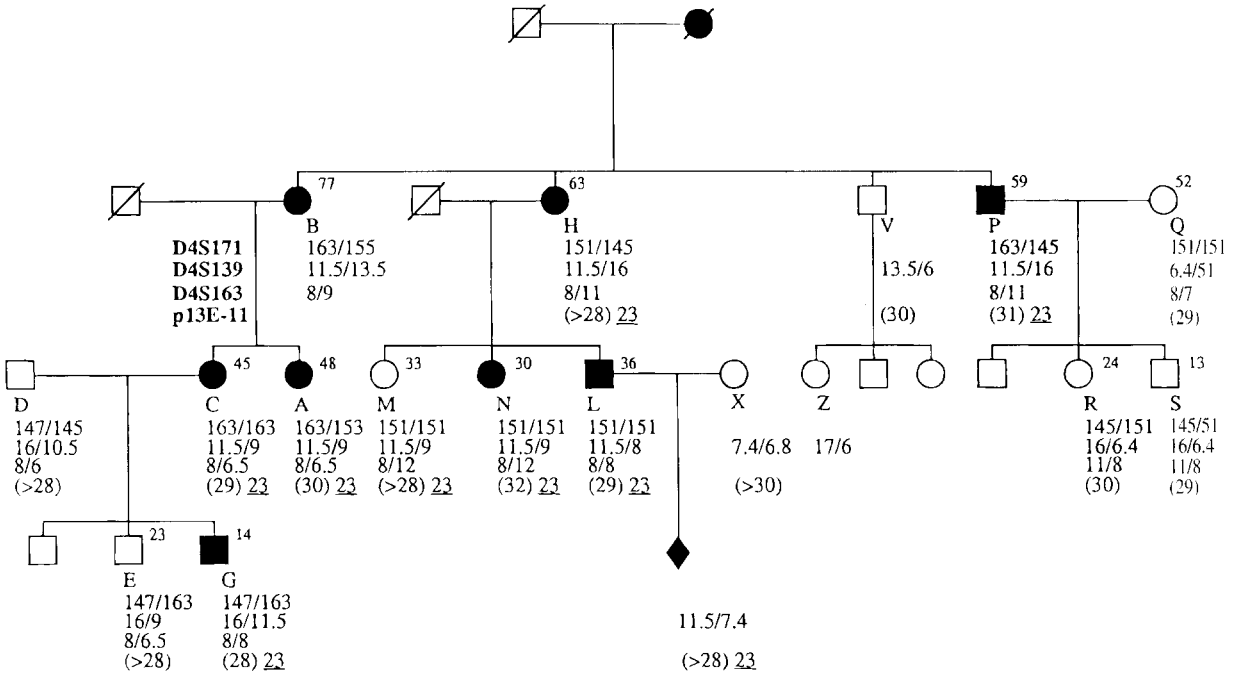

Fig. 2 Chromosome 4q35 haplotypes and p13E-11 DNA fragments in family FSH 01. The disease segregates with the haplotype D4S139 = 11.5 – D4S163 = 8 and is tightly associated with a "small" p13E-11 23-kb fragment *(underlined)*. Larger p13E-11 alleles are in *brackets* and is multiple if preceded by the symbol (>). Subject *M* carries the affected haplotype and the 23-kb "small" fragment, but he is phenotypically normal. DNA analysis was performed on a chorion villi sample *(diamond)* in a couple at risk

These cases will be discussed in detail below, together with the families showing no evidence for linkage between FSHD and 4q35.

Family FSH 01

Figure 2 shows the pedigree of an Italian three-generation family that includes nine affected and six unaffected individuals. The disease segregates with the D4S139 allele 11.5 and the D4S163 allele 8, and this haplotype is tightly associated with a 23-kb p13E-11 fragment. Subject M, aged 33, appears to be a recombinant, since she inherited the affected haplotype and the "small" fragment, but showed no sign of the disease. In this case, it cannot be excluded that she is a nonpenetrant gene carrier.

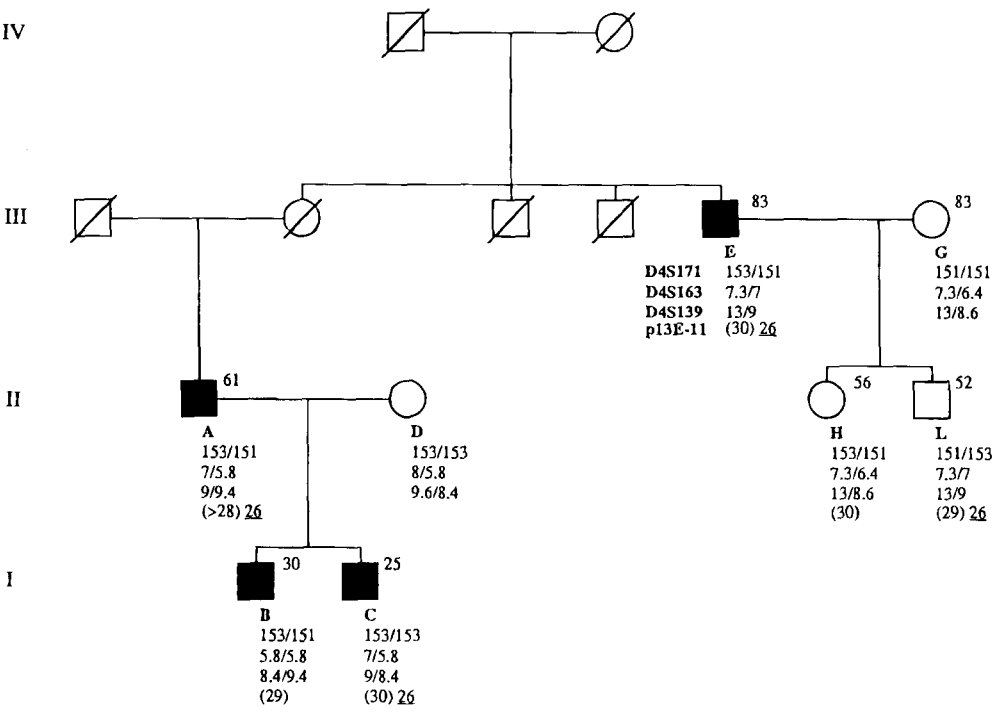

Fig. 3 Chromosome 4q35 haplotypes and p13E-11 DNA fragments in family FSH 03. The disease segregates with the haplotype D4S139 = 9 – D4S163 = 7 and seems to be associated with a 26-kb p13E-11 fragment *(underlined)*. Two recombinants were observed: an affected subject *(B)* carrying the normal haplotype and no "small" fragment, and a normal subject *(L)* carrying the affected haplotype and the 26-kb fragment

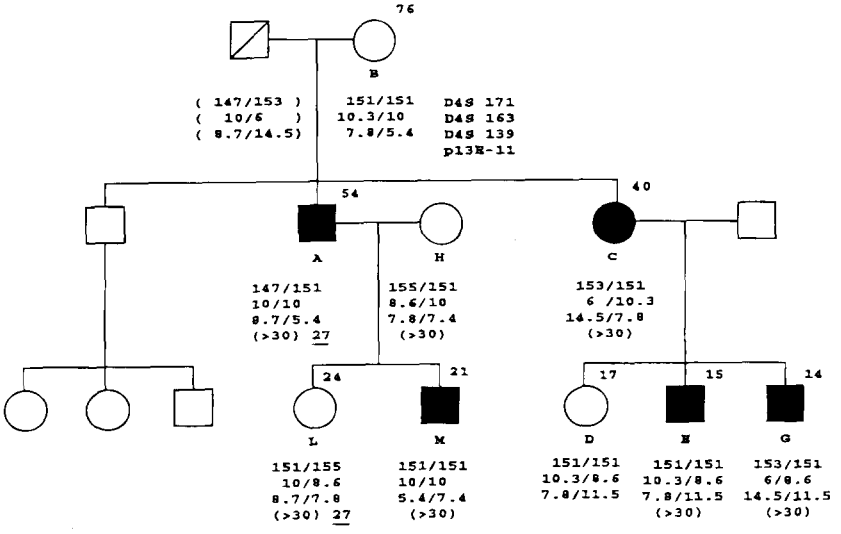

Fig. 4 Chromosome 4q35 haplotypes and p13E-11 DNA fragments in family FSH 12. The disorder does not seem to be linked to chromosome 4q35 markers. The "small" p13E-11 fragment (27 kb, *underlined*) is linked to the other markers, but it does not segregate with FSHD in the affected members of the pedigree. The other p13E-11 alleles are multiple and larger than 30 kb (> 30)

Family FSH 03

Figure 3 shows the segregation of chromosome 4q35 markers in family FSH 03. The disease seems to be inherited with the D4S139 allele 9 and the D4S163 allele 7, and to be associated with a 26-kb shortened fragment (subjects A, C, and E). However, two recombinations seem to have occurred in this family, viz., in subjects 3B and 3L. Subject 3B, affected and carrying the normal haplotype but no "small" fragment, was not aware of his disease status until 28 years of age, when he was submitted to neurological examination in the course of a familial study. He presented a slight weakness of facial and foot extensor muscles, a creatine kinase level twice that of the normal value in several determinations, and an electromyogram with a myopathic pattern. Subject 3L appears to be normal at 52 years of age, although he carries the affected haplotype and the 26-kb fragment. Upon admixture test, this family had a conditional probability of being linked of < 0.05.

Family FSH 12

Figure 4 shows the pedigree of family FSH 12 in which the disease segregates independently from all the tested 4q35 markers as revealed by the admixture test. In addition, we could not identify a "small" fragment cosegregating with FSHD. The only short fragment, 27 kb in size, is present in subject A (affected) and subject L (unaffected)

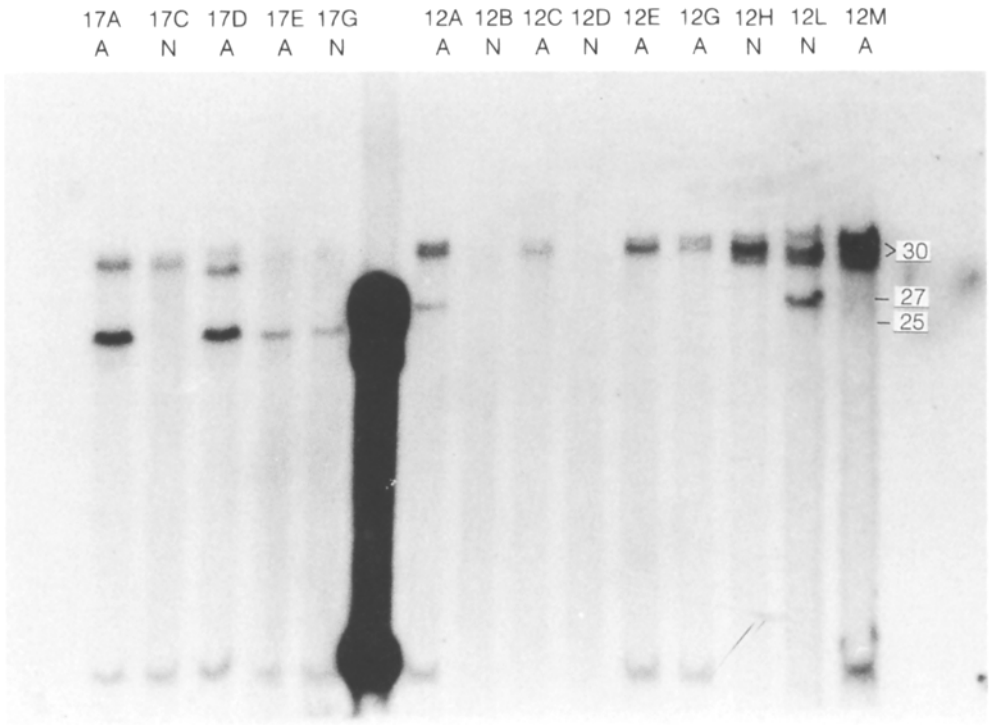

Fig. 5 Hybridization patterns of p13E-11 in two Italian FSHD families. In family FSH 17, a "small" fragment of 25 kb is present in affected *(A)* individuals *(lanes 17A, 17D, 17E)* and is absent in *lane 17C* (*N* normal). Subject 17G *(lane 17G)* carrying the haplotype linked to the affected chromosome and the 25-kb fragment is apparently a recombinant. In family FSH 12 (see Fig. 4 for the pedigree), a "small" fragment of 27 kb does not seem to segregate with the disease, since it is present both in *lane 12A* (*A* affected) and *lane 12L*, (*N* normal), but is absent in other affected members *(lanes 12C, 12E, 12G, 12M)* of the family

Table 4 Clinical and laboratory findings of the affected members of family FSH 12. *F* Facial, *S* scapulohumeral, *L* proximal lower limb, *P* peroneal/anterior tibial, *CK* creatine kinase, *EMG* electromyography, *nt* not tested, *N* normal, ↑ increased

Subjects	Sex	Age (years)	Onset (years)	Muscle groups			CK	EMG	Biopsy
				FSLP	FS	S			
12A	M	54	23	+	–	–	↑	Myopathic	Dystrophic
12C	F	40	35	–	–	+	N	–	–
12M	M	21	–	–	+	–	↑	–	–
12E	M	15	–	–	+	–	nt	–	–
12G	M	14	–	–	–	+	nt	–	–

(Fig. 5). A summary of clinical and laboratory findings in the affected members of the family is reported in Table 4. Subject 12A is the most severely affected with weakness and atrophy of facial and shoulder muscles, bilateral scapular winging, weakness of quadriceps and foot extensor muscles, and steppage gait. His sister (12C) does not present any deficit of facial muscles, but shows scapular winging with atrophy of the pectoralis major, latissimus dorsi, and trapezius. The remaining subjects (12M, 12E, 12G) were examined in the course of the familial study. Subject 12M shows mild weakness of the facial muscles, slight scapular winging, and a moderate atrophy of the latissimus dorsi. Subject 12E exhibits mild weakness of the orbicularis oculi and neck flexors, with a moderate atrophy of the serratus anterior. Subject 12G is affected by mild atrophy of the neck flexors, latissimus dorsi and serratus anterior, with bilateral scapular winging.

Family FSH 17

In this family (Fig. 5), an affected father (17A) has transmitted FSHD to two affected children (17D, 17E) with D4S163 allele 6, D4S139 allele 20, and a p13E-11 fragment 25 kb long. An additional son (17C), unaffected at age 31, inherits opposite alleles at D4S163 and D4S139, and no "small" p13E-11 fragment. However, a fourth son (17G), showing no signs of FSHD at age 29, shares the paternal haplotype and the p13E-11 "small" fragment with his affected sibs. Again, we cannot exclude nonpenetrance of the FSHD gene in this case.

Discussion

We report here data supporting linkage between FSHD and genetic loci D4S171, D4S163, and D4S139 in the majority of Italian families. The physical arrangement of these three markers on 4q35 has been recently determined through radiation hybrid analysis (Winokur et al. 1993). In agreement with the physical map, linkage analysis in our series shows increasing linkage between FSHD and D4S171, D4S163, and D4S139, respectively.

The finding of chromosomal rearrangements in individuals affected by inherited diseases has greatly facili-

tated the search for the relevant mutant genes. The appearance of a novel *Eco*RI fragment detected by the distal probe P13E-11 (D4S810) in sporadic cases of FSHD and the association of the disease with similar "small" fragments in FSHD pedigrees suggest that this probe identifies the site of the mutant gene for FSHD (Wijmenga et al. 1992b). We have confirmed that a "de novo" DNA rearrangement occurs in three sporadic FSHD patients, and that it is associated with the appearance of the clinical and laboratory signs of FSHD disease. In one sporadic case (family 21), the 23-kb fragment is transmitted with the disease to the next generation (submitted for publication), suggesting that the same mechanism is involved in the origin of the "small" rearranged fragments observed in the familial cases of FSHD. In addition, our results show that, in 13 out of 14 Italian 4q35-linked FSHD families, probe p13E-11 detects "small" *Eco*RI fragments in the range 14 kb to 28 kb segregating with the disease. However, in family FSH 18, we were not able to detect any "small" *Eco*RI fragment. In this case, the fragment might comigrate with the larger alleles or be too small to be detected by the methods used.

Two-point linkage analysis between p13E-11 (D4S810) and FSHD support close linkage to the disease locus with a Θ_{max} of 0.001. However, we have observed two putative recombination events between D4S810, D4S139, D4S163, D4S171, and the disease. These occurred in two subjects with no sign of the disease upon thorough clinical examination, although they have inherited a 4q35 haplotype and a p13E-11 "small" fragment identical to their affected relatives. The data reported by Lunt et al. (1988) show that the age of the two subjects is still compatible with a 5% probability of being carriers of the disease. The finding of two such subjects compared with a total of 46 affected subjects in our series is in line with this figure. Therefore, we cannot exclude that nonpenetrance of the mutant FSHD gene might be the cause of the healthy status of these two subjects. The dramatically different implications of these two alternatives on the risk for the offspring indicate extreme caution when counseling these particular individuals.

We have described above the cases of families FSH 03 and FSH12, which were excluded from linkage analysis on the basis of the results of the homogeneity test. Clinical and laboratory findings in these two families meet the International Consortium criteria for diagnosis of FSHD (Padberg et al. 1991). The segregation of 4q35 markers and the failure to detect a FSHD-associated "small" fragment indicate that, in these families, the disease is not linked to chromosome 4q markers. Thus, it appears that genetic heterogeneity is present in the Italian group of FSHD families, and further studies of families with disease showing no linkage to chromosome 4 may prove of great importance for linkage mapping of additional FSHD loci. Therefore, the p13E-11 probe cannot be used to confirm a diagnosis of FSHD disease in the absence of linkage between the 4q35 markers and the disease locus. Evidence for heterogeneity has been provided by a recent paper of Gilbert et al. (1993). In their data, two families out of seven appeared unlinked to the 4q35 region, although the affected individuals met the clinical and laboratory requirements for the diagnosis of FSHD. These results have important implications for clinical investigations, genetic counseling, and prenatal diagnosis, and should contribute to our understanding of the etiology of this neuromuscular disorder.

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References

Di Rienzo A, Felicetti L, Novelletto A, Forteleoni G, Colombo B (1985) Frequency and types of deletional alpha$^+$-thalassemia in Northern Sardinia. Hum Genet 71:147–149

Gilbert JR, Stajich JM, Speer MC, Vance JM, Stewart CS, Yamaoka LH, Samson F, et al (1992) Linkage studies in facioscapulohumeral muscular dystrophy (FSHD). Am J Hum Genet 51: 424–427

Gilbert JR, Stajich JM, Wall S, Carter SC, Qiu H, Vance JM, Stewart CS, et al (1993) Evidence for heterogeneity in facioscapulohumeral muscular dystrophy (FSHD). Am J Hum Genet 53:401–408

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lunt PW, Harper PS (1991) Genetic counselling in facioscapulohumeral muscular dystrophy. J Med Genet 28:655–664

Lunt PW, Noades JG, Upadhyaya M, Sarfarazi M, Harper PS (1988) Evidence against location of the gene for facioscapulohumeral muscular dystropy on the distal long arm of chromosome 14. J Neurol Sci 88:287–292

Mathews KD, Mills KA, Bosch EP, Jonasescu VV, Wiles KR, Buetow KH, Murray JC (1992) Linkage localization of facioscapulohumeral muscular dystrophy (FSHD) in 4q35. Am J Hum Genet 51:428–431

Mills KA, Buetow KH, Xu Y, Ritty TM, Mathews KD, Bodrug SE, Wijmenga C, et al (1992) Genetic and physical mapping on chromosome 4 narrows the localization of the gene for facioscapulohumeral muscular dystrophy (FSHD). Am J Hum Genet 51:423–439

Milner EC, Lotshaw CL, Willems van Dijk K, Charmley P, Concannon P, Shroeder HW Jr (1989) Isolation and mapping of a polymorphic DNA sequence pH30 on chromosome 4 (HGM provisional no. D4S139). Nucleic Acids Res 17:4002

Neuweiler J, Ruvolo V, Baum H, Grzeschik KH, Balasz I (1990) Isolation and characterization of a hypervariable region (D4S163) on chromosome 4. Nucleic Acids Res 18:691

Ott J (1978) A simple scheme for the analysis of HLA linkages in pedigrees. Ann Hum Genet 42:255–257

Ott J (1983) Linkage analysis and family classification under heterogeneity. Ann Hum Genet 47:311–320

Padberg G (1982) Facioscapulohumeral disease. MD thesis, University of Leiden, Leiden

Padberg GW, Lunt PW, Koch M, Fardeau M (1991) Diagnostic criteria for facioscapulohumeral muscular dystrophy. Neuromuscul Disord 1:231–234

Sarfarazi M, Wijmenga C, Upadhyaya M, Weiffenbach B, Hyser C, Mathews K, Murray J, et al (1992) Regional mapping of facioscapulohumeral muscular dystrophy gene on 4q35: combined analysis of an international consortium. Am J Hum Genet 51:396–403

Upadhyaya M, Lunt P, Sarfarazi M, Broadhead W, Farnham J, Harper PS (1992) The mapping of chromosome 4q markers in relation to facioscapulohumeral muscular dystrophy (FSHD). Am J Hum Genet 51:404–410

Weber JL, May PE (1990) Dinucleotide repeat polymorphism at the D4S171 locus. Nucleic Acids Res 18:2202

Weiffenbach B, Bagley R, Falls K, Hyser C, Storvick D, Jacobsen SJ, Schultz P, et al (1992) Linkage analyses of five chromosome 4 markers localizes the facioscapulohumeral muscular dystrophy (FSHD) gene to distal 4q35. Am J Hum Genet 51: 416–423

Weiffenbach B, Dubois J, Storvick D, Tawil R, Jacobsen SJ, Gilbert J, Wijmenga C, et al (1993) Mapping the facioscapulohumeral muscular dystrophy gene is complicated by chromosome 4q35 recombination events. Nature Genet 4:165–169

Wijmenga C, Sandkuijl LA, Moerer P, Boorn N van der, Bodrug SE, Ray PN, Brouwer OF, et al (1992a) Genetic linkage map of facioscapulohumeral muscular dystrophy and five polymorphic loci on chromosome 4q35-qter. Am J Hum Genet 51: 411–415

Wijmenga C, Hewitt JE, Sandkuijl LA, Clark LN, Wright TJ, Dauwerse HG, Gruter AM, et al (1992b) Chromosome 4q DNA rearrangements associated with facioscapulohumeral muscular dystrophy. Nature Genet 3:26–30

Winokur ST, Schutte B, Weiffenbach B, Washington SS, McElligott D, Chakravarti A, Wasmuth JH, Altherr MR (1993) A radiation hybrid map of 15 loci on the distal long arm of chromosome 4, the region containing the gene responsible for facioscapulohumeral muscular dystrophy (FSHD). Am J Hum Genet 53:874–880