

# A closely linked DNA marker for facioscapulohumeral disease on chromosome 4q

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## Abstract

Close linkage of a hypervariable DNA probe on chromosome 4q (pH30, locus *D4S139*) has been found with the locus for facioscapulohumeral disease. Three recombinants were identified in a total of 140 meioses, giving a maximum lod score of 36.77 at a recombination fraction of 0.02. All but two of the families studied proved informative with this probe; all informative families showed evidence of linkage (except one family with a single scorable meiosis), making genetic heterogeneity unlikely from our data. The close linkage and highly informative nature of the probe will make it suitable for clinical application in presymptomatic and prenatal diagnosis. We have also confirmed loose linkage with the marker (*Mfd22*, locus *D4S171*) used to establish the initial assignment of the disorder to chromosome 4.

Facioscapulohumeral disease (FSHD) is one of the commonest of the muscular dystrophies, with a prevalence estimated at around 5 per 100 000.<sup>1-3</sup> It follows autosomal dominant inheritance and is extremely variable both in severity and age of symptomatic onset. Although in most cases weakness first affects the facial muscles in childhood, symptomatic presentation, usually with shoulder girdle weakness,

is often delayed until the second or third decades or even later.<sup>4</sup> The condition is progressive, and although between 10 to 20% of those affected become severely disabled by middle age,<sup>3</sup> others have minimal symptoms throughout life.<sup>2,5</sup> No reliable presymptomatic test for the disorder has been available.

The variability of expression, and the need for accurate presymptomatic detection particularly for genetic counselling, have given impetus to a search for the gene responsible for FSHD. A collaborative group including ourselves has reported exclusion data on a considerable proportion of the genome.<sup>6,7</sup> Although clinical studies of large families favour a single FSHD gene locus,<sup>3</sup> the possibility of genetic heterogeneity has remained, including a possible form of spinal muscular atrophy phenotypically indistinguishable from FSHD.<sup>8</sup> Recently, linkage to a DNA marker on chromosome 4 has been reported in nine Dutch kindreds<sup>9</sup>; while these results found no evidence for genetic heterogeneity, the linkage was insufficiently close (13% recombination) to permit clinical application.

Based on our independent linkage panel of 24 families with FSHD from Great Britain,<sup>7</sup> we report here extremely close linkage of *FSHD* to a new DNA marker on the long arm of chromosome 4, which should allow application in presymptomatic detection and prenatal diagnosis. We also confirm loose linkage with the marker originally reported. Preliminary data from this study have already been presented in brief.<sup>10</sup>

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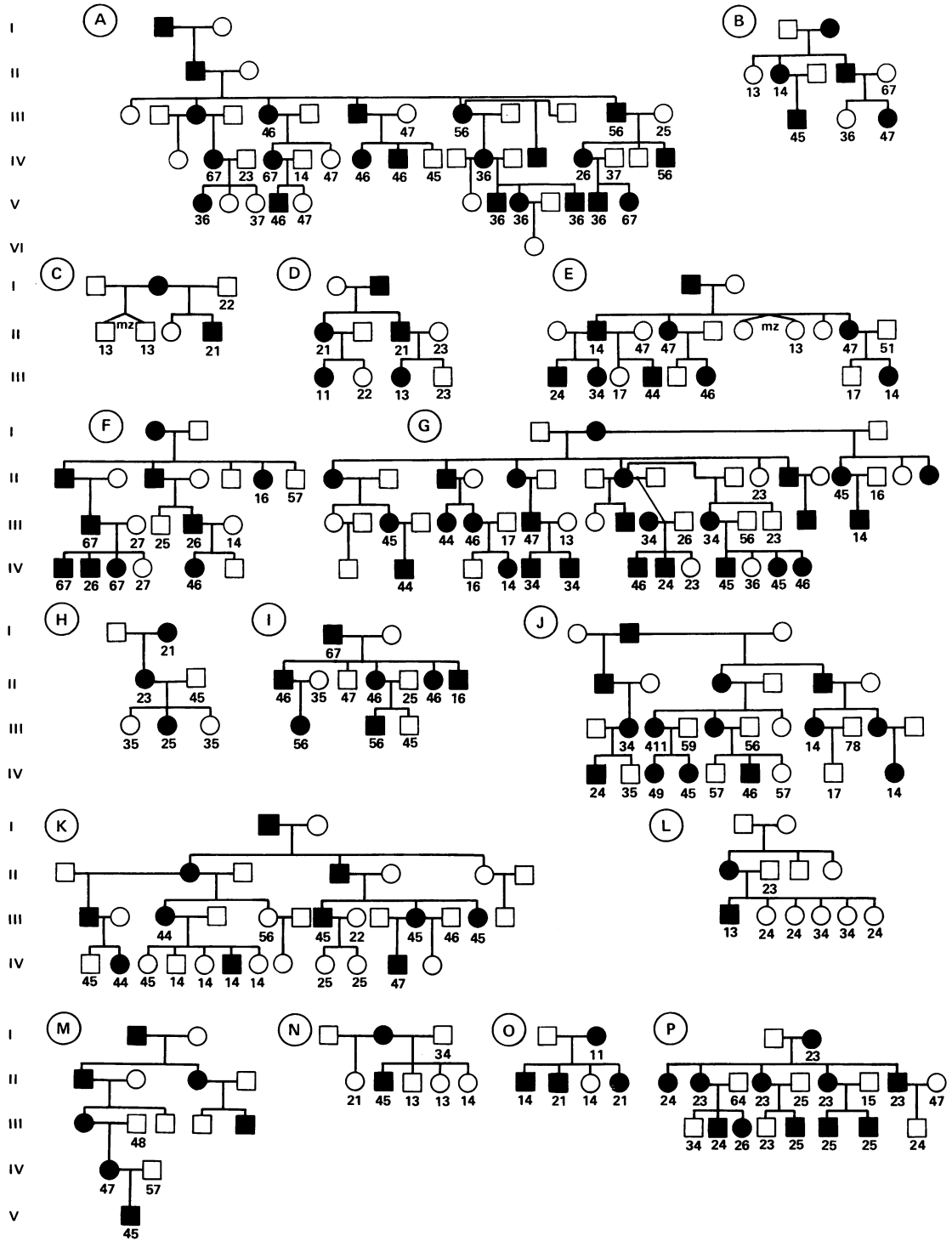
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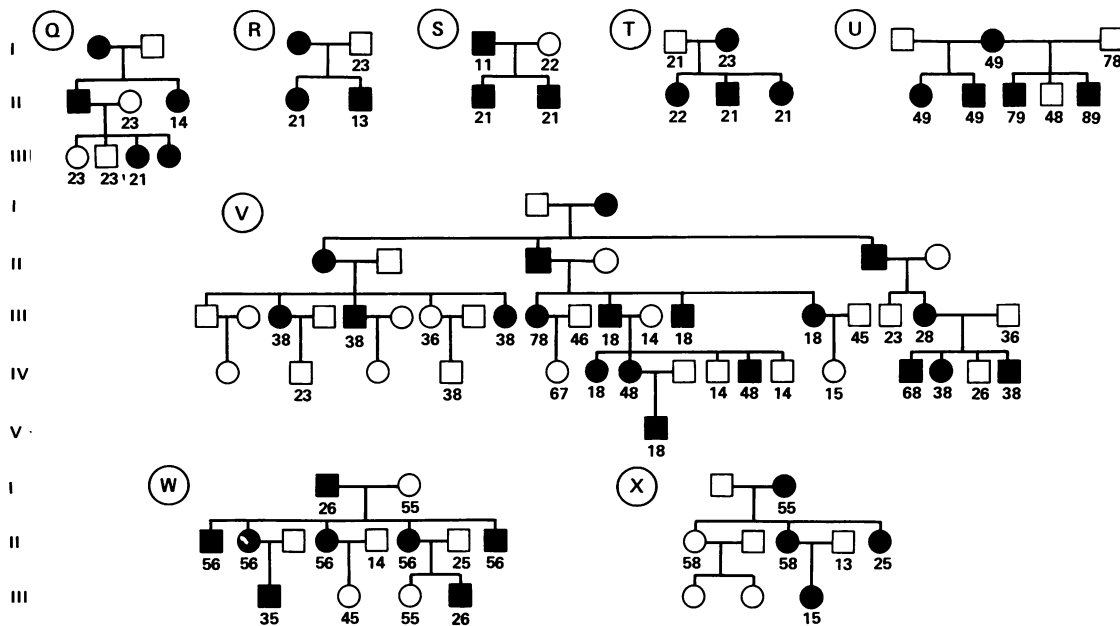
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## Materials and methods

Our family database, which has been reported earlier,<sup>7</sup> comprises 24 families with 151 affected and 248 unaffected subjects, making 194 potentially informative meioses, 34 of which are phase known (figure). A total of 389 out of 399 subjects were assessed clinically by one of us (PL) in their own homes. For inclusion in the linkage panel at least one member from each kindred was required to have facial weakness and to have been diagnosed previously as having facioscapulohumeral muscular dystrophy with supportive electromyogram or muscle histopathology or both. Several of the kindreds had been reported previously by other authors<sup>8,11-13</sup> including one (family 067) in which some subjects





Family pedigrees with genotype data for pH30. Family numbers are as follows: A 001, B 002, C 003, D 004, E 005, F 006, G 009, H 011, I 012, J 025, K 026, L 027, M 028, N 029, O 034, P 035, Q 039, R 041, S 042, T 056, U 062, V 067, W 087, X 091.

had been diagnosed independently as having FSH type spinal muscular atrophy. Fourteen of 60 subjects with minimal clinical signs of disputable significance were included in the linkage analysis with status scored from empirical criteria for combinations of 'hard' and 'soft' signs based on graded weakness in appropriate muscle groups and raised serum creatine kinase levels, as detailed previously.<sup>14</sup> Excluded from analysis were any apparently unaffected subjects under 15 years of age, at which age the penetrance of the FSHD gene is estimated as 70% or less, and 46 other remaining cases of clinical doubt. Subjects from families reported previously<sup>8,11-13</sup> were included in the linkage analysis only after clinical reassessment, but in some cases this allowed updating and extension of the original pedigrees. There were 10 subjects from three families (families 005, 006, and 067) in whom after reassessment we were unable to confirm the previously reported 'affected' status.<sup>8,12</sup> Two of these cases (from family 005) have consistently been included as 'unaffected' in our linkage analysis; the remaining eight cases in whom clinical status remained in doubt, and four others previously reported as 'unaffected' (from families 006 and 067) were consistently excluded.

DNA microsatellite markers *Mfd22* (*D4S171*),<sup>15</sup> which maps to chromosome 4, and *pH30* (*D4S139*),<sup>16</sup> which has been localised to the distal long arm by multipoint mapping, were used in this analysis. DNA was extracted, digested with appropriate enzymes,

fractionated on 0.8% agarose gel by conventional electrophoresis, and Southern blotted onto Hybond N (Amersham). The membranes were hybridised overnight with the DNA probe labelled with <sup>32</sup>P by the random hexanucleotide primed method.<sup>17</sup> The posthybridisation wash was in 2 × SSC, 0.1% SDS and the stringency of washing was increased as necessary. Standard polymerase chain reactions were carried out in a total volume of 25 μl. This contained 20 ng of genomic DNA template, 25 pmol of each oligodeoxynucleotide primer, 200 μmol/l each of dTTP, dGTP, and dCTP, 25 μmol/l dATP, 10 μCi <sup>35</sup>S dATP at 500 Ci/mmol, 50 mmol/l KCl, 10 mmol/l Tris (pH 8.3), 1.5 mmol/l MgCl<sub>2</sub>, 0.01% gelatin, and 1 unit of *Taq* polymerase (Perkin Elmer Cetus). The reaction mixes were overlaid with mineral oil. After an initial denaturation period of five minutes at 94°C they were processed through 25 temperature cycles consisting of 20 seconds at 54°C (annealing), 30 seconds at 72°C (elongation), and 20 seconds at 94°C (denaturation). The last elongation step was lengthened to 10 minutes. Then 10 μl of the amplified DNA were mixed with 4 μl of formamide loading buffer and loaded onto a denaturing polyacrylamide (6%) sequencing gel. Gels were run for three to four hours at 50 to 60 W. They were then fixed and dried before autoradiography. Dideoxy sequencing ladders of M13mp18 were used as size standards.

Two point analysis to determine the maximum likelihood recombination distance and equivalent lod score (*Z*) between the disease locus *FSHD* and

the DNA probe loci was performed using MLINK from version 5.03 of the LINKAGE package.<sup>18</sup> Confidence intervals (CI) were calculated as all values of  $\theta$  for which the lod score was within one unit of the maximum.<sup>19</sup> Unaffected children under 15 years of age were excluded from analysis; no other allowance was made for possible heterozygous status in older unaffected subjects, for whom the risk is 5% or less above the age of 20 years.<sup>14</sup>

## Results

Table 1 summarises our data for the two chromosome 4 markers showing linkage with FSHD. Our data with the DNA microsatellite marker Mfd22 (*D4S171*) support the chromosomal assignment to 4q found by Wijmenga *et al.*,<sup>9</sup> though our maximum recombination fraction of 0.21 (confidence limits 0.10 to 0.37) suggests looser linkage than in their results.

By contrast, the data for probe pH30 (*D4S139*) show extremely close linkage, with a peak lod score of 36.77 at a recombination fraction of 0.02. This hypervariable marker is highly polymorphic, showing 15 identifiable alleles, and with only two families of the 23 studied being uninformative. (DNA samples from one family were contaminated, so this family was excluded from the analysis.)

The data for linkage between the two marker loci given in table 1 show a maximum recombination fraction of 0.16 at a lod score of 3.01 and with wide confidence intervals (0.08 to 0.29).

Table 2 gives the data for *D4S139* and *FSHD* separately by family. Only three crossovers were found in a total of 140 scorable meioses; two occurred in families showing evidence of linkage (families 035 and 087) and one was the single scorable meiosis in family 028, so that our data provide no evidence for genetic heterogeneity. The individual pedigrees are shown in the figure together with genotype data for pH30.

The recombinant event noted in family 028 was in a 15 year old subject whose affected status was questionable, but was scored as 'affected' by the empirical criteria. The recombinant subject in family 087 was also young (14 years) but was undoubtedly 'affected', albeit mildly. In family 035 the recombinant subject was scored as 'unaffected' at the age of 18 years, and again on reassessment at 22

Table 2 Two point linkage analysis between pH30 and FSHD for separate families.

Family No	$\theta$ max	Z max
1	0.00	5.28
2	0.00	1.02
4	0.00	1.09
5	0.00	2.8
6	0.00	2.7
9	0.00	5.9
11	0.00	0.90
12	0.00	2.10
25	0.00	2.59
26	0.00	1.76
27	0.00	1.50
28	0.49	-0.009*
29	0.00	1.20
35	0.08	1.78
39	0.00	0.72
41	0.00	0.20
56	0.00	0.60
62	0.00	8.82
67	0.00	5.91
87	0.11	1.04
91	0.00	0.30

\* (Family 028 at  $\theta = 0.02$ ,  $Z = -1.40$ ).

years after the pH30 typing result. DNA typing with pH30 was informative in 12 of 13 other subjects whose affected status was questionable who were included in the linkage analysis; in all 12 cases the linkage data supported the scoring of status according to the empirical clinical criteria, including one of the subjects (from family 005) scored on reassessment as 'unaffected', but who had appeared in a previous report<sup>12</sup> as 'affected' both on clinical grounds and because of retinal vascular changes; pH30 was uninformative in the other similar case.

## EXCLUSION DATA

Until June 1990 the chromosomal localisation of FSHD was unknown, though individual and pooled exclusion data had been published.<sup>6-8,20-23</sup> Such data remain relevant in determining whether genetic heterogeneity exists, and our additional unpublished data for other chromosomes are summarised in table 3. Of particular note is the positive lod score of 1.56 at a recombination fraction of 0.10 for the oestrogen receptor locus (*ESR*) on chromosome 6q. Because of the proximity of this to the autosomal dystrophin locus,<sup>24</sup> the latter appeared to be an important candidate gene for FSHD, but analysis of a polymorphism at this locus (using probe BSM7), in

Table 1 Two point linkage analysis between FSHD and the two DNA marker loci (*D4S171* and *D4S139*).

Probe	Locus	Linkage to	$\theta$ max	Z max	95% CI
Mfd22	<i>D4S171</i>	<i>FSHD</i>	0.21	1.98	0.10 - 0.37
pH30	<i>D4S139</i>	<i>FSHD</i>	0.02	36.77	0 - 0.05
	<i>D4S139</i>	<i>D4S171</i>	0.16	3.01	0.08 - 0.29

Table 3 Lod scores for linkage between other DNA loci and FSHD.

Locus	$\theta$ (cM)	Lod score	Chromosomal location	Probe
<i>CD2</i>	0.10	-2.04	1p13	
<i>REN</i>	0.10	-2.04	1p32	pHRnES1.9
<i>D2S3</i>	0.10	-5.2	2q35-q37	p5-1-30,p5-2-96
<i>D3S6</i>	0.27	0.24	3pter-q21	DR82
<i>D3S5</i>	0.10	-8.69	3p21-qter	DR-2
<i>D4S81</i>	0.10	-1.12	4p16.3	HDA-RB1.6
<i>D4S10</i>	0.10	-2.75	4p16.3-p16.2	G8
<i>D4S93</i>	0.10	-1.54	4pter-p15.1	G6
<i>MT2P1</i>	0.10	-3.011	4p11-q21	pHM6
<i>FGA/FGB</i>	0.10	-5.06	4q28	pAF1, pH1B2
<i>D5S1</i>	0.39	0.17	5	L1.7
<i>D5S37</i>	0.22	0.34	5q21	pi227
<i>D5S71</i>	0.10	-1.72	5q21-q22	C11p11
<i>ESR</i>	0.10	1.56	6q24-27	pOR3
<i>MYB</i>	0.30	0.66	6q22-23	pHM2.6
<i>ARG1</i>	0.13	0.13	6q23	G16B
<i>PLG</i>	0.10	-6.17	6q26-q27	
<i>SOD2</i>	0.30	0.12	6q21	phMnS0D4
Autosomal dystrophin	0.29	0.16	6q24	BSM7
<i>MET</i>	0.10	-6.19	7q31-q32	pmetH, pmetD
<i>LPL</i>	0.10	-2.66	8p22	Lipoprotein lipase
<i>TG</i>	0.10	-1.41	8q24	pCHT16/8.0
<i>D9S3</i>	0.10	-2.84	9q	DR6
<i>CDC2</i>	0.10	-2.04	10q21.1	CDC2-H(PDB 231)
<i>RBP3</i>	0.10	0.27	10q11.2	H41RBQ
<i>HRAS1</i>	0.30	0.49	11p15.5	pTB-2
<i>INT2</i>	0.24	0.51	11q13	SS6
<i>D11S24</i>	0.21	0.57	11q13-q23	E4b-TGH2
<i>D11S84</i>	0.10	-3.37	11q22	p2-7-1D6
<i>ETS1</i>	0.31	0.34	11q23.3	c-ets
<i>PAH</i>	0.10	-3.21	12q22-q24.2	phPAH247
<i>F8VWF</i>	0.23	0.81	12pter-p12	pVWF750
<i>A2M</i>	0.10	-2.10	12p13	A2 macroglobulin
<i>COL2A1</i>	0.10	-3.51	12q14.3	CosHcoll
<i>D13S5</i>	0.26	0.32	13q22-q34	pHUB8
<i>IGHG1</i>	0.10	-1.6	14q32.3	24BRH
<i>GM</i>	0.40	0.22	14q32	
<i>D15S1</i>	0.10	-4.73	15q14-q21	pM51-14
<i>D15S2</i>	0.37	0.15	15q11	
<i>HBA1</i>	0.10	-4.37	16pter-p12	globin
<i>APRT</i>	0.10	-3.76	16q24	Huap15
<i>D17S71</i>	0.10	-3.47	17p11-q11	pA10-41
<i>D17S36</i>	0.35	0.21	17q	CRI-946
<i>D18S3</i>	0.10	-5.89	18p11.3	B74
<i>D18S7</i>	0.35	0.39	18q11.1-q11.2	V11A8
<i>D18S8</i>	0.38	0.30	18q21.3	V11E10
<i>D18S11</i>	0.10	-6.77	18q23	p25
<i>APOC2</i>	0.20	-1.38	19q12-q13.2	
<i>D19S9</i>	0.15	0.66	19q12-q13.3	IJ2
<i>PKCG</i>	0.10	-8.53	19q13.2-qter	
<i>D20S5</i>	0.10	-2.10	20p12	pBR12.12
<i>D20S6</i>	0.10	-2.455	20p12	pD312
<i>D21S11</i>	0.20	0.185	21q11.2-q21	pW236B
<i>IGLV</i>	0.28	0.117	22q11.1-q11/2	V33H3A
<i>SISR12</i>	0.10	-0.847	22q12.3-q13.1	pDGFB

conjunction with Dr Kay Davies, showed frequent recombination. It should also be noted that those families contributing most to the positive score at the oestrogen receptor locus (families 035, 006, and 087) also show positive scores for *D4S139*, suggesting that chance rather than locus heterogeneity has been responsible for the apparent evidence for linkage at this locus.

### Discussion

Our results not only confirm the assignment of the gene for FSHD to the long arm of chromosome 4,

but for the first time provide a tightly linked and highly informative marker locus that is suitable for presymptomatic and prenatal diagnosis. We have already shown that the variability in manifestation and age at onset of FSHD is such as to make molecular genotyping clinically important in confirming or excluding the presence of the gene in subjects at risk,<sup>14</sup> while the severity in a proportion of affected subjects is such as to make prenatal diagnosis an important option for some family members.<sup>3</sup>

*D4S139* is likely to prove a particularly suitable marker for clinical use in view of its highly poly-

morphic nature, rendering almost all families informative, and the closeness (2% recombination) of the linkage. It is likely that the current confidence limits (0 to 5%) will be further narrowed in the near future as further data are obtained.

Our data give no support for genetic heterogeneity in FSHD, two of the only three recombinants observed being in families showing clear evidence of linkage to 4q; the third recombinant was the single scorable meiosis in the family and involved a subject whose affected status was questionable. Since our family panel contains 24 kindreds of different severity, including childhood onset,<sup>3</sup> and also three kindreds, branches of which were diagnosed elsewhere as 'FSH spinal muscular atrophy',<sup>38</sup> it seems likely that a single genetic locus is responsible for FSHD. Nevertheless, until more data are available we would urge caution in clinical application in families that are too small individually to provide evidence for linkage, and would also recommend that, for the present, families should be analysed as a whole so that the presence of linkage can be confirmed.

Our finding of a closely linked marker will also be helpful in assessing the significance of associated non-muscular features of FSHD. Both nerve deafness<sup>25</sup> and retinal changes<sup>12</sup> have been reported in association with the disorder, but it is currently not clear how consistent or universal such abnormalities are, nor whether they can be used in presymptomatic detection in the absence of neuromuscular abnormalities, particularly since we have found one case in which the DNA linkage analysis supports our scoring of 'unaffected' clinical status in preference to a previously reported retinal vascular abnormality. The closeness of the linkage with *D4S139* should clarify these points in other cases.

The precise localisation of the FSHD gene on chromosome 4q is currently under study, as is its relationship to other genetic markers in the region. These markers include the DNA probe EFD139,<sup>26</sup> the factor XI gene (FII),<sup>27</sup> and the autosomal breakpoint of the X;4 translocation found in a patient with Duchenne muscular dystrophy.<sup>28</sup> Study of the relationship of FSHD to these and further loci should determine flanking markers for the disorder and should also contribute to our understanding of the detailed map of this region of chromosome 4.

There are currently no obvious candidate genes for FSHD known on the basis of either its chromosomal localisation or the nature of the neuromuscular defect; the autosomal dystrophin locus has been conclusively excluded by the results described above. However, the accurate localisation that now exists for FSHD will make the assessment of future candidate genes feasible and will also allow the techniques of physical mapping to be applied to the characterisation of a well defined and relatively restricted chromosomal region.

Finally, the existence of close and continuing collaboration between the groups involved in research on FSHD, something that has already proved of great value in the initial localisation of the gene, should be of even greater help in subsequent steps towards isolation of the gene itself.

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