A genetic linkage study of facioscapulohumeral (Landouzy-Déjérine) disease with 24 polymorphic DNA probes

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SUMMARY From analysis of DNA polymorphisms in a panel of 455 subjects from 25 families with facioscapulohumeral (Landouzy-Déjérine) disease, we have found no evidence for close linkage of the disease at 24 different genetic loci, including one from a candidate chromosomal region. Added to previous data, our results provide direction for future collaborative linkage studies.

Facioscapulohumeral (Landouzy-Déjérine) disease (FSHD), with a prevalence estimated at 5/100 000,^{1 2} is arguably one of the more common inherited neuromuscular disorders. Clinical onset is usually in childhood with facial weakness, but first symptomatic presentation with shoulder girdle weakness is often delayed until the teens, early adulthood, or later.³ Progression of weakness occurs, especially in the proximal muscles of the upper limbs, but often also involves truncal, peroneal, and/or more proximal lower limb muscles, so that up to 20% of FSHD heterozygotes require a wheelchair by middle age.⁴ The question of whether Landouzy-Déjérine disease

is a single genetic entity, or whether forms of spinal muscular atrophy, mitochondrial myopathy, and muscular dystrophy can each present with a facioscapulohumeral syndrome currently remains unresolved.⁵ Chromosome regional localisation of the FSHD gene (at least in some families) would help to resolve this question, would be an important step towards eventual understanding of the pathogenesis of the disease, and would have immediate application in predictive and prenatal testing where appropriate.

In the absence of any previous reports of isolated chromosomal anomaly with FSHD, or of cosegregation with any other dominantly inherited disorder, gene localisation depends on establishing linkage with a protein or DNA polymorphism. Early

Received for publication 9 March 1989.
Accepted for publication 31 March 1989.

TABLE 1 *Lod scores from early linkage studies in FSHD.*

Locus	Location*	Reference	Recombination fraction (θ)			
			0.1	0.2	0.3	0.4
Rh	1p36.2-p34	6	Not closely linked			
		7	Not informative			
		8	-1.33	-0.58	-0.23	-0.0
Fy	1q22-q23	8	-1.56	-0.64	-0.24	-0.05
		6	Not closely linked			
MNS	4q28-q31	7†	-1.18	-0.41	-0.08	0.03
		8	-0.17	-0.28	0.15	0.06
		6	-1.35	0.13	0.07	0.09
ABO	9q34.1-q34.2	6	Not closely linked			
		7†	0.22	0.13	0.10	0.02
		8	0.15	0.25	0.24	0.09
Jk	18q11.1-q11.2	8	-0.89	-0.38	-0.15	-0.04
Se	19q12-q13	7†	Small positive scores			
PI	22q11.2-qter	7†	Small positive scores			
PTC	—	8	-0.67	-0.25	-0.09	-0.02
		6	Not closely linked			

*Gene locations are from *Human Gene Mapping 9*⁹ and from *New Haven Human Gene Mapping Library* (No 4, HGM9.5 issue).

†Clinical status reassessed in present study.

studies⁶⁻⁸ provided a small amount of data, but with no evidence to suggest linkage to any of six blood group loci (table 1). Close linkage (recombination fraction $[\theta] < 0.1$) to 13 loci for protein and blood group polymorphisms was excluded in 10 Dutch families by Padberg *et al.*,¹⁰ who found a suggestion of linkage to *Gm* (localisation 14q32.3) with a maximum lod score ($Z_{\max}$) of 1.438 at $\theta=0.2$. Further studies have failed to support this possible localisation of FSHD close to the IGHG locus.¹¹⁻¹³

Using a panel of 399 subjects from 24 kindreds with FSHD, our initial studies¹³ excluded the possibility of close linkage with *IGHG* (*Gm-FSHD*: $Z=-2.42$ at $\theta=0.2$). With the inclusion of DNA samples from one large Dutch family, we have continued our linkage study by systematic exclusion of several chromosomal regions as possible locations for FSHD through testing for linkage to RFLPs, using DNA probes selected for maximum potential

information content and for availability in our laboratory or from colleagues. We report here analysis of the first 24 probes tested, including one mapping to chromosome 18 within a region recently found by cytogenetic analysis to be deleted in an isolated child with features of FSHD.

Materials and methods

Our linkage panel consisted of 399 subjects (151 affected, 248 unaffected) from 24 families who were assessed clinically by one of us (PL) in their own homes, and 56 subjects (23 affected, 33 unaffected) from one large Dutch family (DNA samples kindly donated by G Padberg). Dominant inheritance was proven in all pedigrees. Excluding unaffected children, there are 194 potentially informative meioses of which 34 could be phase known. At least one member from each kindred was required to have facial weakness and to have been diagnosed previously as having 'facioscapulohumeral (FSH) muscular dystrophy' with supportive electromyogram or muscle histopathology or both. In three kindreds included in the analysis, other members had been diagnosed independently as having 'FSH type spinal muscular atrophy'. A few subjects with minimal clinical signs of disputed significance were scored as affected or unaffected according to empirical combinations of 'hard' and 'soft' signs, as detailed previously.¹³ Any remaining cases of doubt, including two with markedly raised serum creatine

TABLE 2 Age dependent penetrance of FSHD.

Age group (y)	Proportion of FSHD heterozygotes clinically detectable (penetrance)
0-4	<0.05
5-9	0.21
10-14	0.58
15-19	0.86
20+	0.95

Figures are derived from assessment of the segregation ratio in subjects selected for minimum bias from 15 families.¹³

TABLE 3 Lod scores for linkage between 24 DNA loci and FSHD.

Locus	Location ⁹	Recombination fraction (θ)					
		0.01	0.05	0.10	0.20	0.30	0.40
D4S10	4p16.3-p16.2	—	-5.10	-2.75	-0.85	-0.16	0.03
D5S37	5q21	-1.00	0.61	0.94	0.88	0.50	0.13
D5S71	5q21-q22	-6.19	-3.53	-2.38	-1.22	-0.57	-0.19
Met D/Met H	7q31-q32	-10.79	-4.79	-2.51	-0.78	-0.23	-0.09
TG	8q24	-0.92	0.27	0.60	0.62	0.40	0.15
HRAS1	11p15.5	-10.78	-4.25	-1.83	-0.07	0.38	0.32
ETS1	11q23.3	0.75	0.57	0.42	0.30	0.23	0.11
PAH	12q22-q24.2	-5.31	-2.02	-0.78	0.14	0.38	0.29
IGHG1 ¹³	14q32.2	-6.23	-3.02	-1.63	-0.48	-0.08	0.00
Gm ¹³	14q32.2	-26.21	-12.40	-6.92	-2.42	-0.71	-0.11
HBA1	16p13.3	-14.76	-6.88	-3.90	-1.52	-0.59	-0.19
APRT	16q24	-15.78	-8.82	-5.90	-3.08	-1.50	-0.54
D17S71	17p12-p11.2	-23.09	-11.19	-6.62	-2.87	-1.27	-0.44
D17S36	17q	-27.41	-11.26	-5.42	-1.29	-0.19	-0.02
D18S3	18p11.3	-10.28	-2.33	0.10	1.07	0.76	0.29
D18S7	18q11.1-q11.2	-29.04	-13.58	-7.60	-2.64	-0.74	-0.07
D18S8	18q21.3	-57.10	-26.02	-14.08	-4.40	-0.77	0.29
D18S11	18q23	-31.40	-14.30	-7.81	-2.72	-0.94	-0.37
APOC2	19q12-q13.2	-16.24	-7.28	-3.92	-1.39	-0.52	-0.18
D19S9	19q12-q13.2	0.36	1.36	1.47	1.11	0.60	0.19
D20S5	20p12	-6.31	-2.52	-1.22	-0.35	-0.10	-0.00
D20S6	20p12	-11.94	-5.84	-3.33	-1.21	-0.40	-0.10
D21S11	21q11.2-q21	-2.32	-0.50	0.05	0.28	0.19	0.06
IGLV	22q11.1-q11.2	-0.15	0.94	1.15	0.94	0.53	0.16
SISR12	22q12.3-q13.1	-6.89	-3.10	-1.79	-0.91	-0.51	-0.19

kinase but normal clinical examination, were excluded.

Extraction of DNA from peripheral blood, preparation of probes, and characterisation of RFLPs was by standard techniques.¹⁴ Southern blotting was onto Gene Screen Plus (Du Pont) and Hybond N (Amersham) nylon filters for repeated hybridisations with ³²P labelled DNA probes.

Lod scores were calculated by MLINK,¹⁵ after exclusion of unaffected children under the age of 15 years (except for establishing haplotypes), and with age weighting of older asymptomatic subjects to allow for age dependent risk of heterozygote FSHD status (table 2).

Results

Table 3 gives the physical localisation and lod scores with sexes combined for the RFLPs used in the present study, and includes, for completeness, our previous results¹³ with *Gm* and *IGHG*. None of the combined lod scores was indicative of linkage ($Z_{\max} > 3.0$), although in one large family a maximum lod score of 1.87 at $\theta = 0.15$ was obtained with one probe (D18S3). Small combined positive scores are noted on chromosome regions 5q, 11p, 11q, 17q, 18q, 19q, and 21q.

Discussion

Our results further extend chromosome regions excluded as probable sites for the location of *FSHD* and contribute to a current exclusion map.¹⁶ While acknowledging the possibility of genetic heterogeneity, we have included three families where clinical diagnoses of FSH muscular dystrophy and FSH type spinal muscular atrophy have been made independently in different members, as there is little evidence to support these being separate genetic conditions²; also, at a recent international workshop on Landouzy-Déjérine disease, it was recommended that the most efficient initial approach to gene mapping would be to construct a mutual exclusion map from linkage results for these two diagnoses combined.⁵

Chromosome 18 probes were included after the report of an isolated case of a young girl with features of FSHD, deafness, and retardation who had a de novo deletion of 18q22-qter (A C Berry, 1987, personal communication). Small positive lod scores were obtained with probes mapping more proximally on 18q, but there was convincing evidence against linkage with *D18S11*, which maps to the deleted region. Linkage to *Jk* (18q11.1-q11.2) had also previously been tested and small negative scores obtained.^{8, 10} The one large family giving a positive

score with D18S3 had previously been assessed clinically,¹⁷ and we thank Professor Edwards for subsequent referral. Our reassessment identified no distinctive clinical or biopsy features to suggest that this family had a unique FSH syndrome, and we conclude that the results on chromosome 18 are likely to be fortuitous, although they indicate a need for further studies. In two families, possible cosegregation between familial adenomatous polyposis coli and FSHD was noted (L P Rowland, 1988, personal communication). Linkage between polyposis coli and chromosome marker C11p11 defined by locus *D5S71* has been reported, $\theta = 0.0$, $Z = 3.26$.¹⁸ In view of this finding, our family panel was typed with two long arm chromosome 5 markers, C11p11 and $\pi 227$, both mapping to 5q21-q22. A small positive score ($\theta = 0.10$, $Z = 0.94$) with probe $\pi 227$ was obtained but there was a negative lod score at all recombination fractions with probe C11p11. Linkage studies with other long arm chromosome 5 markers (LI.4, LI.7) are under analysis.

Following our reassessment of clinical status in the members of one family, 35 years after the original report,⁷ and pooled with other reports,^{8, 10} we calculated a maximum lod score for linkage with *MNS* (4q28-q31) of 0.80 at $\theta = 0.3$. This result, together with the small positive scores obtained with probes mapping to 10, 11, 17q, 19q, and 21q, suggests other chromosomal regions for further study. Future collaboration with other groups is planned in order to avoid duplication of results and to maximise the efficiency of further linkage studies for mutual benefit.

We are grateful to the members of the various families for their patient cooperation, to Dr George Padberg for DNA samples from one large family, and to the other clinicians who have referred families for study. We thank the following for the gift of DNA probes: J Gusella (D4S10, D21S11), N Spurr (D5S37, D5S71), P O'Connell (Met D, Met H), G van Ommen (TG), G Carter (HRAS1), S Kittur (ETS1), S Woo (PAH), D Cox (IGHG), J Old (HBA), P J Stambrook (APRT), D Barker (D17S71), K Stevens (D17S36), J L Mandel (D18S3), G Thomas (D18S7, D18S8), G Bruns (D18S11), D Shaw (APOC2, D19S9, D20S6), L Meredith (D20S5), J C Kaplan (IGLV), and T Robbins (SIS). This study was funded by the Muscular Dystrophy Group of Great Britain.

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