Facioscapulohumeral disease

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Chapter 5

Laboratory studies

5.1. Introduction

As many patients as possible were studied with the use of routine laboratory procedures in order to establish the diagnosis in the kindreds as firmly as possible. These procedures included the examination of the serum creatine kinase (CK) activity, needle electromyography (EMG) of several muscles, and biopsies of muscles for histological and histochemical staining. Open biopsies (25) as well as needle biopsies (5) (Edwards 1980) were performed. In several possibly informative kindreds blood and saliva were collected for linkage studies.

5.2. Serum creatine kinase activity

Using automated sample analysers serum creatine kinase (CK) activity was determined in 77 patients with FSHD, and in 39 non-affected sibs (19 males, 20 females), all with one affected parent. The average CK activity in the latter group was 27.9 U/l for both sexes (see Table 5.1.).

Table 5.1. Average serum creatine kinase (CK) activity in 39 non-affected sibs; values in U/l

	Number of patients	Average CK activity	Range	Upper limit of normal
Males	19	21.7	2 - 44	50
Females	20	33.8	0 - 364	35
Total	39	27.9	0 - 364	

Two unaffected females had elevated CK activities. One patient revealed several bruises on physical examination for which she had no good explanation. Her serum CK activity was 364 U/l. Serum CK-MB activity was not detectable in her case. The other patient had a serum CK activity of 53 U/l. If the former patient is disregarded, the average serum CK activity for females would be 16.4 U/l (range 0 - 53) and, for all non-affected sibs 19.1 U/l.

The average serum CK activity was 84.5 U/l in 77 affected sibs (Table 5.2.), and the average value in males (106.4 U/l) was approximately double that in females (53.6 U/l). The asymptomatic patients had a lower level than the symptomatic patients. Twenty-eight patients (15 males, 13 females) had normal CK levels (36%). Normal values were predominantly found in the more advanced stages of the disease.

Table 5.2. Serum creatine kinase (CK) activity in 77 patients with FSHD; values in U/l

	Symptomatic patients	asymptomatic patients	all patients
Males No.	40	5	45
Average CK	107.7	96.8	106.4
Female No.	21	11	32
Average CK	56.4	48.2	53.6
Total No.	61	16	77
Average CK	90.0	63.4	84.5

Table 5.3. shows the relation between serum CK activity and age. Patient M III 8 had a serum CK activity of 1265 U/l at the age of nine years. He was already severely affected at that time. The high CK level is felt to reflect the high rate of progression in his case. The next highest value was 214 U/l. All other values were below 200 U/l. The decline of the serum CK activity with age

is statistically significant (P = 0.0001 Rank Regression Analysis). A similar decline is observed if the relation between the CK activity and the duration of the disease is considered (Table 5.4). This decline with duration is also statistically significant (P = 0.001, Rank Regression Analysis).

The relation between the serum CK activity and the stage of the disease is demonstrated in Table 5.5. If patient M III 8 is disregarded, because of the unusual rapid progression of the disease, the average serum CK-activity in stage 4 would be 38.0 U/l. The low level of CK activity in stages 5 and 6 could reflect both the diminished muscle mass and the immobility of the patients in this stage.

Table 5.3. Relation between serum creatine kinase (CK) activity and age in years in 77 patients with FSHD; values in U/l

Age in years	0 - 9	10 - 19	20 - 29	30 - 39	40 - 49	50 - 59	60 - 69	70 - 79	80 - 89
Males No	1	4	9	11	5	9	4	2	-
Average CK	1265	71.0	112.0	87.6	118.4	52.1	31.0	42.0	-
Females No	-	3	7	7	4	5	5	-	1
Average CK	-	115.0	51.0	59.7	32.5	51.6	38.6	-	14
Total No	1	7	16	18	9	14	9	2	1
Average CK	1265	89.9	85.3	76.8	80.2	51.9	35.2	42.0	14

Table 5.4. Relation between serum creatine kinase (CK) activity and duration of the disease in years in 61 symptomatic patients with FSHD; values in U/l.

Duration	0 - 9	10 - 19	20 - 29	30 - 39	40 - 49	50 - 59
Males No	6	11	7	10	5	1
Average CK	279.7	93.8	112.1	54.0	28.6	55.0
Females No	5	6	6	1	3	-
Average CK	85.0	57.7	43.3	23.0	36.3	-
Total No	11	17	13	11	8	1
Average CK	192.1	81.1	80.4	51.2	31.4	55.0

Table 5.5 Serum creatine kinase (CK) activity related to the stage of the disease in 77 patients with FSHD; values in U/l

	stage 1	stage 2	stage 3	stage 4	stage 5	stage 6
No of patients	20	14	33	4	3	3
Average of CK	64.6	72.2	75.9	344.8	25.3	25.3
Range of CK	10 - 168	23 - 176	11 - 214	23 - 1265	25 - 26	14 - 44

In summary, only a general statement can be made: serum CK activity is only slighty raised in FSHD, in most cases does not exceed four times the upper limit of normal, and declines with age and duration of the disease.

Serum CK-MB isoenzyme activity was measured in 16 patients by ion-exchange column chromotography (Mercer, 1974). It turned out that slightly elevated CK values yielded no detectable CK-MB fractions. In 11 patients (eight males and three females) with CK values above 100 U/l the average CK-MB fraction was 4.4% (range 0 - 6.2%) of the total CK activity. Values were considered normal up to 3%. These observations support previous reports (Klapdor et al., 1977) that the CK-MB fraction rarely exceeds 6% of the total serum CK activity in patients with neuromuscular disorders. The origin of the elevation of the serum CK-MB fraction in patients with FSHD is not quite clear. Silverman et al. (1976) and Zweig et al. (1980) suggested that the MB isoenzyme is of skeletal origin. It certainly cannot be concluded that the cardiac muscle is involved in FSHD.

Serum CK activity was also examined in four sibs younger than ten years. All had normal values. Only one sib (A VII3), five years old, was suspected to be affected on clinical grounds. He had a CK value of 25 U/l. These persons have not been restudied to establish the predictive value of serum CK examination. Walton and Gardner-Medwin (1981) apparently observed no predictive value of the serum CK levels in healthy children of parents with FSHD.

5.3. Genetic Linkage

Genes are considered to be linked if their loci lie in proximity to each other on the same chromosome. The closer the distance between the loci, the smaller the chance that crossing-over between the two homologous chromosomes occurs during gametogenesis. The distance between the loci is expressed in centimorgans. One centimorgan means a recombination frequency of 1%. Large families, in which many generations can be examined are most informative in linkage studies. Also the loci that are studied need to show sufficient heterogeneity in the population. Rarely are all these requirements met. In practice one has to rely on the combined information of several smaller families. Statistical analysis of data, mostly performed with the aid of a computer program, gives the probability of linkage at a given fraction of recombination (θ). These probabilities are expressed in logarithms and are called lod-scores (logarithm of odds). The logarithmic form allows the addition of the results of various studies. If the lod-scores are +3 or higher, linkage is assumed. If the scores are -2 or lower, linkage at the given genetic distance is highly improbable. No definite conclusion can be reached if the scores are between -2 and +3.

We studied possible linkage of the locus of the gene for FSHD with 35 different marker genes. The ten most informative and cooperative families (kindred A - J) participated in this study. We collected blood and saliva from 62 affected and 58 non-affeced members, including 17 non-affected parents.

The blood groups ABO, MNS, P, Rhesus (RH), Kell (K), Lutheran (LU), Duffy (FY) and Kidd (JK) were determined. Phosphoglucomutase (PGM1), 6 phosphogluconate dehydrogenase (PDG), adenylate kinase (AK1), esterase D (ESD), glutamate-pyruvate-transaminase (GPT), superoxide dismutase (SOD1), phosphoglycolate phosphatase (PGP), glyoxalase (GLO), NADPH-diaphorase (DIA2), acid phosphatase (ACP1) adenosine deaminase (ADA), were investigated in lysates of erythrocytes. Pseudo-cholinesterase (CHE2), α 1-antitrypsin (PI), haptoglobin (HP), group-specific component (GC), complement factor (C3), transferrin (TF) and the

Table 5.6 Lod-scores for linkage between the loci for FSHD and several genetic markers at different recombination fractions (θ).

	ABO	MNS	P	RH	K	LU	FY	JK
0.01	- 8.468	- 9.469	0.167	-23.456	-6.773	0.029	-7.513	-1.083
0.10	- 2.316	- 0.762	0.182	- 5.916	-3.124	0.016	-2.665	-0.508
0.20	- 0.887	0.700	0.141	- 1.891	-1.795	0.007	-1.151	-0.232
0.30	- 0.387	0.734	0.077	- 0.494	-0.988	0.004	-0.455	-0.089
0.40	- 0.181	0.276	0.021	- 0.082	-0.426	0.001	-0.147	-0.016

	PGD	PGM1	AK1	ESD	GPT	GLO	ACP1	ADA
0.01	- 2.548	- 4.742	- 1.222	- 8.843	-7.372	-12.622	-7.389	-7.764
0.10	- 0.825	- 1.379	- 0.321	- 2.611	-1.542	- 3.861	-1.745	-2.920
0.20	- 0.247	- 0.544	- 0.124	- 1.085	-0.318	- 1.626	-0.540	-1.920
0.30	- 0.048	- 0.193	- 0.043	- 0.390	0.004	- 0.631	-0.194	-1.469
0.40	0.004	- 0.039	- 0.009	- 0.095	-0.009	- 0.168	-0.097	-0.633

	PI	HP	GC	C3	TF	AM	KM	GM
0.01	-15.577	- 3.600	- 9.613	- 0.848	0.068	- 0.606	-2.285	-2.716
0.10	- 5.715	- 1.542	- 2.328	- 0.507	0.071	- 0.282	-0.956	1.277
0.20	- 2.500	- 0.779	- 0.731	- 0.258	0.074	- 0.048	-0.341	1.428
0.30	- 1.108	- 0.319	- 0.156	- 0.108	0.056	0.016	-0.043	0.889
0.40	- 0.292	- 0.077	0.001	- 0.027	0.023	0.013	0.053	0.252

	HLA-A	B	C	D	AMY1
0.01	-16.964	-22.767	-18.346	-13.725	-2.763
0.10	- 4.120	- 6.721	- 5.179	- 3.892	-0.835
0.20	- 1.253	- 2.575	- 1.764	- 1.347	-0.345
0.30	- 0.185	- 0.747	- 0.348	- 0.301	-0.126
0.40	0.126	- 0.019	0.127	0.057	-0.029

immunoglobulin marker genes AM, KM and GM were studied. Human Leucocyte Antigens (HLA) were typed, and in saliva Amylase (Amy 1) and the proline-rich saliva proteins (PR, PA and DB) were determined. Statistical analysis was carried out with the use of the LIPED program (Ott, 1974).

The scores obtained are represented in Table 5.6. SOD1, DIA2, CHE2, PR, PA, and DB were not informative in these kindreds: their scores were 0 and have been omitted. None of the marker genes studied gave a clear indication for linkage with FSHD. For the polymorphic marker genes ABO, RH, K, FY, ESD, GLO, ADA, GC, PJ and HLA linkage with FSHD at a recombination fraction of 0.10 could be excluded. The highest score obtained was 1.428 for GM at θ 0.20. This score, together with the score of 1.277 at a recombination fraction of 0.10, suggests that there is a possibility that the genes for FSHD and for GM are linked, assuming only one locus for FSHD. More data on independent families will be required to confirm or refute this possibility. Recent studies proved that the locus for the GM system, which codes for the constant region of the heavy chains of IgG immunoglobulins, is situated on the short arm of chromosome 14 (Croce et al., 1979; Shander et al., 1980; Cook et al., 1981). Linkage is known to exist between the GM locus, and PI locus which codes for α 1-antitrypsine. A peak lod-score of 20.75 is found for a GM-PI recombination fraction estimate of 0.26 (Gedde-Dahl et al., 1981). Since the scores for PI and FSHD were all negative in our material, the gene for FSHD - if it were to be found linked with GM - would have to be on the other side of GM.

5.4. Electrophysiological studies

Concentric needle electromyography (EMG) was performed in 31 patients (17 males, 14 females). In all patients at least five relevant muscles were sampled. These muscles were the left deltoid, the right biceps brachii, the right quadriceps femoris, the left gastrocnemius and the right extensor digitorum brevis in 26 patients. In five patients several other muscles were chosen. In 16 patients more than five muscles were sampled.

In all patients the motor nerve conduction velocity was measured in the right peroneal nerve. In nine patients additional motor nerves were studied.

Table 5.7 shows that four patients (two males, two females) had normal electromyograms. One of the males had undergone another EMG two years prior to our examination, which was said to show myopathic patterns at that time. We do not feel a need to introduce new terms, as has been argued in Chapter 2, and will continue to use the terms myopathic patterns and myopathic EMG in those cases where a mixed or interference pattern of brief, small and often polyphasic action potentials is found. We are well aware that certain neurogenic lesions could lead to similar findings (W.K. Engel, 1975).

Table 5.7 Electrophysiological studies in 31 patients with FSHD

			Males	Females	Total
EMG	Patients with normal studies		2	2	4
	Patients with myopathic features only		11	12	23
	Patients with myopathic and neurogenic features		4	0	4
Motor conduction velocity of the right peroneal nerve		≥ 40 m/sec	15	12	27
		< 40 m/sec	2	2	4

EMG of some or all muscles studied revealed a myopathic pattern and no neurogenic findings in 23 patients (Table 5.7). Four male patients, all of different families, had a myopathic pattern on EMG of several proximal upper extremity muscles, while EMG of leg muscles demonstrated neurogenic characteristics. One of these patients (Q III 5) had diabetes mellitus and clinical findings of a polyneuropathy, which had not been present in the past. The polyneuropathy could account for the neurogenic findings on EMG of his lower extremities. His sisters both had a typical picture of FSHD and a normal sensory examination (see kindred Q). The right quadriceps femoris muscle in patient C II 6 showed spontaneous fibrillation potentials and a mixed to interference pattern on maximal voluntary muscle contraction with polyphasic action potentials, and individual action potentials of long duration and amplitudes of 1000 microvolts. The motor nerve conduction velocities were within normal limits. EMG in patient N III 10 revealed spontaneous fibrillation potentials in the right tibialis anterior muscle with a single to mixed pattern on maximal contraction, polyphasic potentials and amplitudes up to 1000 microvolts. The left gastrocnemius muscle and the right extensor digitorum brevis muscle showed a mixed pattern with many polyphasic potentials. The duration of the potentials was not quantified, but the pattern was interpreted as compatible with a neurogenic lesion. The motor nerve conduction velocity of the right peroneal nerve was normal. Also in patient P III 2 (38 years old) a mixed pattern, with many polyphasic potentials and potentials of long duration and increased amplitude was recorded in the right serratus anterior muscle (2000 microvolts) and in the right extensor digitorum brevis muscle (2000 microvolts). The left anterior tibial muscle revealed a single pattern on maximal contraction with polyphasic potentials of long duration and amplitudes of 1000 microvolts, while the right anterior tibial muscle showed a myopathic pattern with potentials of 200-300 microvolts. Fibrillation potentials were observed in the right serratus anterior muscle in this patient.

EMG of the orbicularis oris and orbicularis oculi muscles were performed in two patients (A IV and I VI 11), showing

myopathic features in both.

High frequency (pseudomyotonic) discharges were recorded in patients M III 8 (gastrocnemius), P III 1 (tibialis anterior), and S III 6 (quadriceps femoris).

The motor nerve conduction velocity of the right deep peroneal nerve exceeded 39 m/sec in 27 patients. Patient Q III 5 had a polyneuropathy and a conduction velocity of 30 m/sec. No proper response could be elicited in what was left of the extensor digitorum brevis muscle in patient A IV 3. Two patients (F III 3 and N III 12) showed a conduction velocity of 39 m/sec. The meaning of these values is debatable. At the time of examination the patients were 70 and 50 years old respectively. Two patients (J III K, 78 years old, and J IV 10, 46 years old) demonstrated conduction velocities of 31 m/sec and 23 m/sec respectively. Repeated examination with control of the temperature of the extremities yielded values of 45 m/sec in both patients.

The conduction of other motor nerves tested in nine patients yielded no abnormalities, with the exception of a slight reduction of the conduction velocity in the right (39 m/sec) and in the left (38 m/sec) posterior tibial nerves in patient P III 2. The motor conduction velocities in the peroneal nerves in this case were normal. A previous examination had shown a reduced motor nerve conduction velocity in the right median nerve. Repeated examination revealed normal values.

Our observations strongly suggest that, if properly executed, measurements of motor nerve conduction velocities in FSHD will reveal no abnormalities.

It can be concluded that routine EMG in FSHD shows myopathic features in the majority of cases. Occasionally (in 10% of our cases, i.e. in three out of 30 cases), neurogenic features such as abundant fibrillation potentials, in combination with - often polyphasic - potentials of increased duration and amplitude, may be observed in some of the muscles sampled. The neurogenic features may be present in the upper as well as in the lower extremities.

5.5. Muscle biopsies

Muscle biopsies have been performed in patients of all kindreds, with the exception of kindred Q (see description of the kindred in chapter IV). In patient O III 6 the biopsy was said to have been compatible with a myopathy, but the slides could not be reviewed. Patient H V 16 had undergone a biopsy of the right quadriceps femoris. Only paraffin embedded slides had been made. These were reexamined and showed an increased variation of fibre diameter with hypertrophic fibres (up to 140 micron in diameter) and an advanced endomysial fibrosis and fatty infiltration. Prior to his examination at the neuromuscular clinic of the University of Amsterdam patient F III 3 had undergone biopsies of the left pectoralis major and the left anterior tibial muscles. These biopsies were reported to have been compatible with a myopathy.

We studied 30 muscle biopsies obtained from 28 patients. Histological as well as histochemical preparations have been made in all instances. Haematoxylin and eosin (HE), NADH-tetrazolium reductase (NADH-TR) and myofibillar adenosine triphosphatase (ATP-ase) stains have been made in all cases. Modified Gomori's trichrome, periodic acid Schiff, lactic dehydrogenase and succinic dehydrogenase stains were available in most instances. Occasionally acid phosphatase, oil red O, and other stains have been used.

Six of the 30 biopsies (20%) revealed no abnormalities (Table 5.8). Therefore the diagnosis of FSHD in kindred D rests on non-morphological criteria (see description of this kindred). A similar situation was present in kindred Q, in which no patient had a muscle biopsy. Early in the course of the disease a normal biopsy is particularly found in muscles that are not (yet) involved on clinical examination such as the deltoid of quadriceps femoris muscles. They confirm the experience of physical examination that muscle involvement may be localized in FSHD.

Twenty-four biopsies from 23 patients revealed abnormalities. F IV1 had two biopsies. The biopsies have been examined using the criteria outlined in Table 5.9. Figure 5.1 represents the percentages of the abnormalities found.

Table 5.8. Muscle biopsies without abnormalities in patients with FSHD

S	III	11:	R. deltoid
G	III	20:	L. deltoid
H	V	20:	R. deltoid
I	VI	11:	R. quadriceps femoris
N	III	12:	L. quadriceps femoris
P	III	2:	L. triceps brachii

FIGURE 5.1: FINDINGS IN 24 MUSCLE BIOPSIES OF 23 PATIENTS WITH FSHD

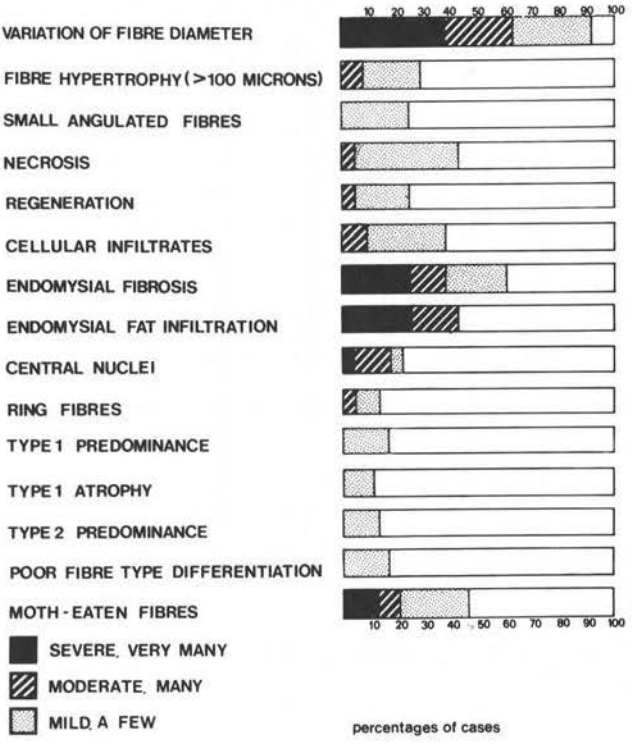

Table 5.9. Resul

Patient	AIV3	AV14	BIII1	BIII6	CII6	CIII1	EIV1	FIII3	FIV1		GIII
Site of biopsy[1]	QL	QL	TAL	TAL	QL	TAR	QR	QL	QL	TR	QR
Variation of fibre diameter[2]	+++	+	+++	+++	+++	++	+	++	-	+	+++
Fibre hypertrophy[2] (>100 u)	-	-	-	+	++	++	+	-	-	-	-
Small angulated fibres[2]	-	+	-	-	-	-	-	-	-	-	-
Necrosis degeneration[2]	-	-	-	+	-	+	+	+	-	-	-
Regeneration[2]	-	-	-	++	-	+	+	-	-	-	+
Cellular infiltrations[2]	-	-	-	-	+	++	++	+	-	-	-
Fibrosis	-	-	++	+++	+++	+	+	+++	-	-	+++
Fat (endomysial)[2]	++	-	++	+++	+++	-	-	+++	-	-	+++
Central nuclei[3]	-	-	++	-	++	+	-	-	-	-	-
Ring fibres[2]	-	-	-	-	-	-	-	+	-	-	-
Type 1 predominance[4]	-	-	-	-	+	+	-	-	-	-	-
Type 1 atrophy[4]	-	+	-	-	-	-	-	-	-	-	-
Type 2 predominance[4]	-	-	-	-	-	-	-	-	-	-	-
Uniform enzyme activity[4]	-	-	+	+	-	-	-	-	-	-	+
Moth-eaten fibres[2]	+++ (G)	+	+	-	++ (G)	+ (G)	-	+++ (G)	+	-	+

1. Q: quadriceps femoris; TA: tibialis anterior;
T: triceps brachii; D: deltiodeus; B: biceps brachii;
R: right; L: left.

2. -: absent; +: mild, a few; ++: moderate, many;
+++: severe, very many.

f 24 muscle biopsies in 23 patients with FSHD.

VI11	JIV10	KIV2	LIV42	LIV44	LIV44	LV84	MIII8	NIII10	PIII1	RII6	SIII2	SIII6
L	QL	QR	QR	BL	QR	QR	QL	BL	BR	QL	TAR	DR
+	+	–	++	++	+	+	+++	+++	+++	+	+++	++
	–	–	–	+	–	–	–	–	–	–	+	–
	–	+	+	–	–	+(G)	–	–	+	+(G)	–	–
	–	–	+	+	–	–	++	–	+	–	–	+
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	–	–	–	–	–	–	–	–	–	–	+	–
	–	–	–	–	–	–	+	+++ (G)	–	–	++	–

3. –: absent; 3–10%; ++: 10–20%; +++: 20% or more

4. –: absent; +: present.

G indicates: observed in groups.

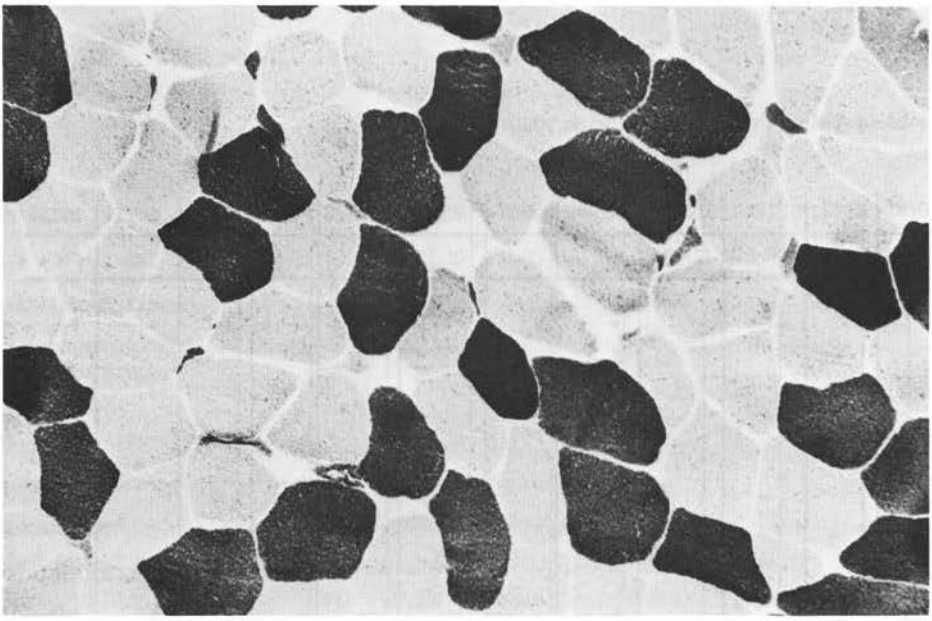
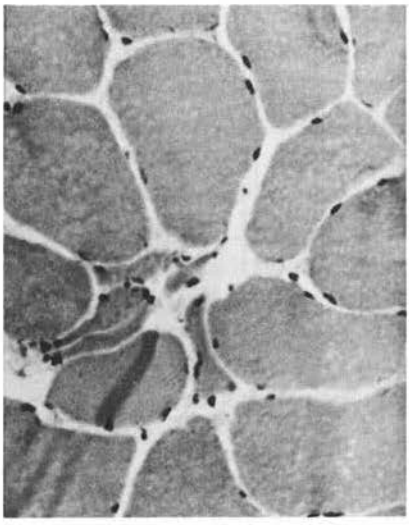
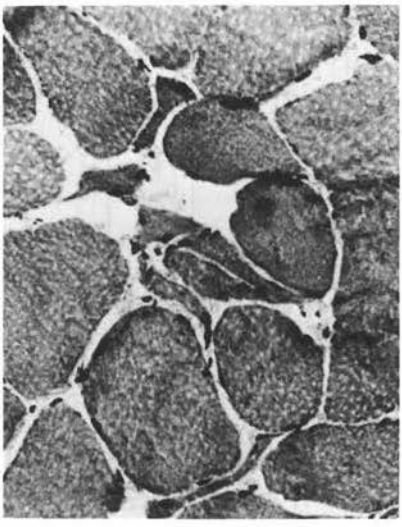


Figure 5.2. Patient LV84: small angulated fibres. Routine ATP-ase x 400.





Figures 5.3. and 5.4. Patient LV84: small groups of atrophic fibres. HE X 400.

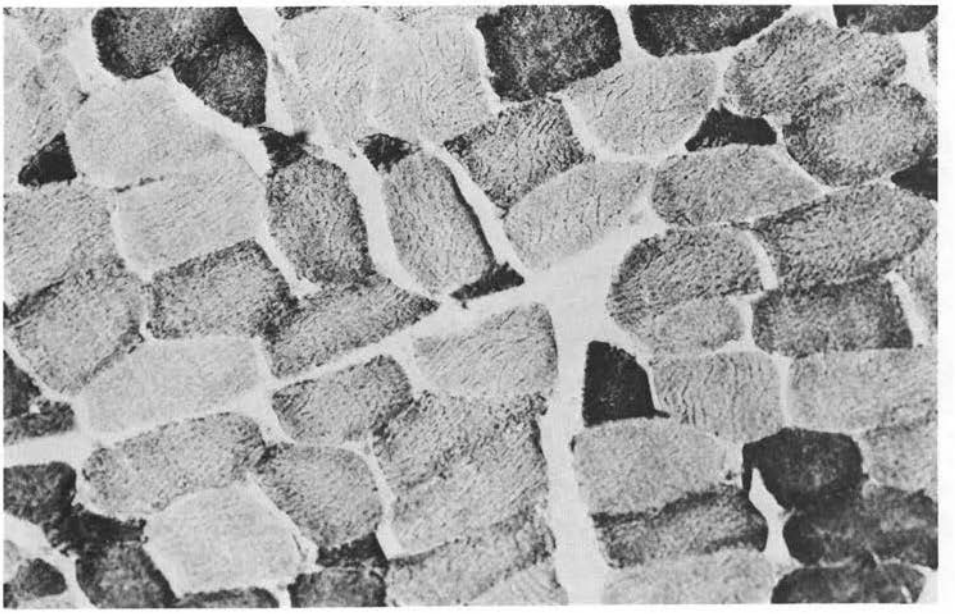


Figure 5.5. Patient A V14: atrophic type 1 fibres. NADH-TR x 400.

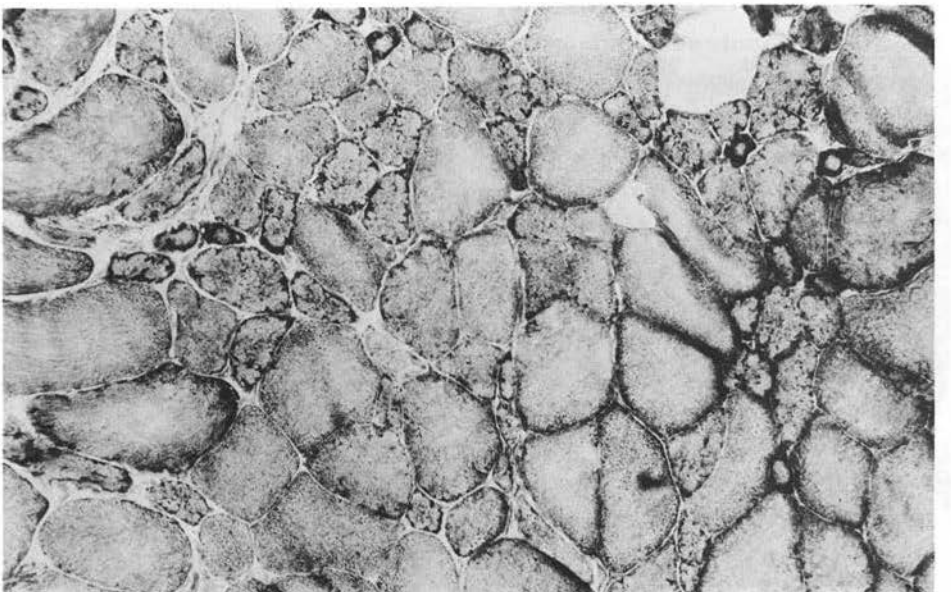


Figure 5.6. Patient F III 3: groups of moth-eaten fibres. NADH-TR X 400.

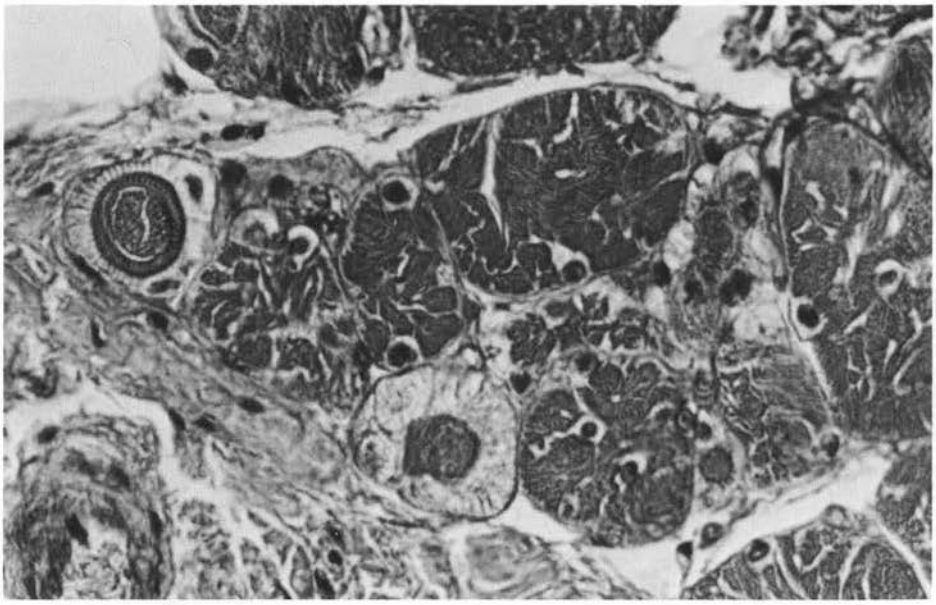


Figure 5.7. Patient N III 10: sarcoplasmic masses and ring fibres. HE X 1000.

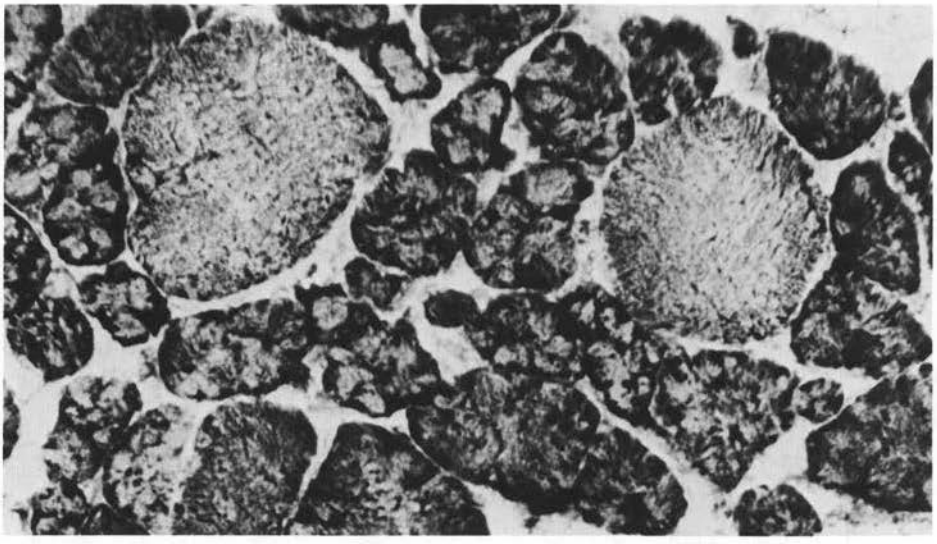


Figure 5.8. Patient N III 10: moth-eaten fibres in clusters. NADH-TR X 1000.

An increased variation of fibre diameter was present in most cases (92%). Fibres were measured using a calibrated ocular. Hypertrophic fibres, i.e. fibres with a diameter exceeding 100 microns were found in one-third (33%) of the cases. Small angulated fibres were present in six biopsies (25%) (Figure 5.2). In four of these biopsies the small fibres occurred in small clusters of three or four cells (Figures 5.3 and 5.4). Larger groups of atrophic cells were not observed in these biopsies. Signs of degeneration such as necrosis and phagocytosis were present in eight biopsies (33%). The biopsy with the most necrotic fibres was the one of patient M III 8, who indeed exhibited the most rapid progression of the disease of all patients. Signs of regeneration, such as basophilic fibres with vesicular nuclei and prominent nucleoli, were remarkably inconspicuous. Small cellular infiltrations, occasionally rather frequently present, were never as extensive as reported in the literature (Munsat et al., 1972). No relation was apparent with complaints of muscle pains, but the pain was invariably located in the shoulder region, where a biopsy was rarely taken. Endomysial fibrosis (58%) and fatty infiltration (42%) were rather common in this material. Most of these patients were seen in a rather advanced stage of the disease. Ring fibres were observed in three patients (12.5%). Sarcoplasmic masses were present in the biopsy of patient N III 10. Fibre splitting was observed in three biopsies (J IV 10, K IV 2 and S III 2).

The histochemical stains were used for fibre typing, for the study of the organisation of the fibres in the biopsy, and for the identification of morphological peculiarities such as moth-eaten fibres. Predominance of fibre type 1 was observed in four biopsies (17%). Selective atrophy of type 1 fibres was observed in two biopsies (8%). Figure 5.5. shows the NADH-TR stain of the biopsy in patient A V 14. The atrophic fibres are type 1 fibres, as was affirmed by the ATP-ase stain. Predominance of fibre type 2 was present in three (12.5%) biopsies.

Poor fibre type differentiation (Dubowitz and Brooke, 1973) was observed in four biopsies. This phenomenon is often reported in Duchenne muscular dystrophy but it is ill-explained. Van

Wijngaarden and Bethlem (1973) observed uniform enzyme activity in 27 out of 32 biopsies of patients with benign infantile spinal muscular atrophy. They suggested that uniform enzyme activity might be the result of the neurogenic lesion in these patients. The uniform enzyme activity in these two conditions might not be the result of the same mechanism. In two of our biopsies (G III 17 and S III 2) vague suggestions of fibre type differentiation were present in other than ATP-ase and NADH-TR stains.

Moth-eaten fibres have been observed in 11 biopsies (46%). This frequency is remarkable and in agreement with previous observations (Bethlem at al. 1973). They varied from small fibres, scattered through the biopsy, to large fibres in clusters. In five biopsies they occurred in groups (Figure 5.6.). The reasons for this arrangement are obscure. A vasogenic lesion might be an explanation although morphological abnormalities of the small vessel walls have not been observed. However perivascular cellular infiltrations were present in four of those five biopsies. Another explanation for the grouped arrangement can be found in postulating a neurogenic lesion. The aberrant myofibrils and the sarcoplasmic collections in the moth-eaten fibres have been elegantly demonstrated by Bethlem et al. (1973): they resemble at an ultrastructural level the ring fibres with the sarcoplasmic masses known from histological examinations. Patient N III 10 demonstrated both features (Figures 5.7. and 5.8.).

In summary, judged by pathological criteria, it can be said that both the nature and the degree of muscle involvement may be quite variable. Most abnormalities suggested a myopathic condition. The low degree of necrosis and the even lower degree of regeneration were remarkable and compatible with the slowly progressive course of the disease. The amount of cellular infiltrations was impressive at times, but infiltrations were not present in all biopsies.

Definite neurogenic findings such as type grouping, distinct group atrophy, and target fibres, have never been observed, but several features such as small groups of atrophic fibres, small angulated fibres, and clusters of moth-eaten fibres suggest that

neurogenic factors might play a role in FSHD. One or more of these features were present in 11 (46%) out of 24 abnormal biopsies.

5.6. Discussion

Physical examination and family studies (Chapter 4) suggested that the patients in kindreds A-S suffered from the same disorder. Linkage studies (5.3.) did not give evidence for linkage between the locus for the gene of FSHD, and the loci of any of the 35 genetic markers used.

The serum creatine kinase (CK) activity revealed a rather uniform pattern in these patients (5.2). Elevation of the CK activity rarely exceeded four times the upper limit of normal. Normal values were obtained in approximately one third of our patients, and particularly in the more advanced stages of the disease. Determination of the CK activity might be helpful in the occasional case when physical examination leaves doubts whether someone is affected or not. A marked CK elevation does not rule out FSHD but suggests also to consider disorders such as polymyositis. In most instances determination of the serum CK activity is not very helpful in the differential diagnosis of FSHD, because all disorders to be considered are able to give CK elevations similar as in FSHD.

In several instances needle EMG (5.4.) and histological and histochemical examination of muscle biopsies (5.5.) revealed features considered to be of neurogenic origin. We will discuss the results in each kindred in order to demonstrate that the neurogenic abnormalities occur in some members of the families and not in others of the same families. It is inferred that neurogenic features in EMG and muscle biopsy are part and parcel of this disease.

Kindred A demonstrates the importance of histochemical staining in muscle biopsies. Groups of small fibres in the HE stain proved to be moth-eaten fibres in A IV 3. In patient A V 14 the small fibres represented selective type 1 atrophy. In both

patients EMG showed myopathic features. Myotonia was not observed.

Patients B III 1, and B III 6 both showed myopathic features on EMG. Poor fibre type differentiation was present in the biopsies of the anterior tibial muscles in both these patients. This might be related to the site of the biopsy, as patient S III 2 presented a similar picture. In the other cases, where the anterior tibial muscle was biopsied (C III 1), type 1 predominance was found, a feature reported to occur frequently in biopsies of these muscles (Mercelis et al., 1981).

Both patients in family C showed moth-eaten fibres in groups and no small angulated fibres. In patient C III 1 EMG was performed in another hospital and reported to be without abnormalities: he was not reexamined. Patient C II 6 had neurogenic features on EMG of his quadriceps femoris muscle.

In patient D III 11 EMG showed myopathic features. Biopsy of his deltoid muscle revealed no abnormalities.

Myopathic features were present both on EMG and in the biopsy of patient E IV 1.

Patient F III 3 demonstrated many moth-eaten fibres in groups (Figure 5.6.), myopathic features on EMG and a reduced conduction velocity of his right peroneal nerve (39 m/sec). EMG in his daughter (F IV 1) revealed no abnormalities.

The two brothers in family G were studied in more detail. Both had myopathic features on EMG. G III 17 showed myopathic features on histological examination of the biopsy of the right quadriceps femoris muscle: histochemistry revealed poor fibre type differentiation. The biopsy in his brother G III 20 was normal.

EMG and histological examination in patient H V 16 suggested a myopathy. The EMG and muscle biopsy in his cousin (H V 20) revealed no abnormalities. Patient I VI II had myopathic features on EMG and muscle biopsy similar to patient J IV 10.

In patient K IV 2 both angulated fibres and fibre splitting were present in the biopsy of his right quadriceps muscle. EMG revealed no abnormalities.

In family L, EMG studies suggested a myopathic condition in

L IV 44, L IV 45 and L V 84, while the muscle biopsy revealed myopathic abnormalities in patients L IV 42, L IV 44 and L IV 45. Patient L V 84 demonstrated numerous small angulated fibres, occasionally occurring in a small group (figures 5.2., 5.3., and 5.4.).

In patient M III 8, both EMG and muscle biopsy suggested a myopathic condition.

The motor conduction velocty of the right peroneal nerve in patient N III 12 was 39 m/sec. EMG revealed myopathic features. A biopsy of the left quadriceps femoris muscle was normal. Her brother, patient N III 10, was observed by others to show fasciculations in several shoulder girdle muscles. When he was examined by us, neither fasciculations nor myotonia were observed. His calf muscles were well developed and slightly paretic. EMG of upper extremity muscles showed myopathic findings, but EMG of the anterior tibial muscle showed spontaneous fibrillation potentials and a single to mixed pattern on voluntary contraction with polyphasic action potentials and single potentials up to 1000 microvolts. A biopsy of the left biceps muscle in this patient revealed a marked variation in fibre diameter, ring fibres, sarcoplasmic masses and numerous central nuclei. Large numbers of moth-eaten fibres could be demonstrated (Figures 5.7. and 5.8.). This biopsy could have been compatible with myotonic dystrophy but neither myotonia nor other physical characteristics suggesting this disorder were present.

The muscle biopsy in patient O III 6 could not be re-examined: it was said to have shown myopathic changes.

On EMG P III 2 demonstrated neurogenic features in some muscles in his arms and legs. Biopsy of the left triceps muscle revealed no abnormalities. EMG in P III 1 suggested a myopathy. Biopsy of the right biceps revealed small angulated fibres and type 2 predominance. Similar findings were present in patient R II 6.

The neurogenic features on EMG of the leg muscles in patient Q III 5 could be explained by the diabetic polyneuropathy from which he suffered. EMG of the arm muscles showed a myopathic pattern. A muscle biopsy was refused.

Two patients of family S (S III 2 and S III 6) had a myopathic pattern on EMG. Muscle biopsies revealed myopathic abnormalities on histological examination in both patients. Histochemical studies revealed poor fibre type differentiation in patient S III 2 in whom the anterior tibial muscle was biopsied. In patient S III 6 some fascicles suggested type 2 predominance, while a normal pattern was present in other fascicles. Such experiences emphasize that chance factors are involved in taking a biopsy.

Several features of the muscle biopsies have been put forward in section 5 to be due possibly to neurogenic lesions. Such lesions could account for the neurogenic features on EMG in patients with FSHD. The most convincing features, suggesting a neurogenic factor in FSHD, are small groups of atrophic fibres, small angulated fibres and individual amplitudes larger than 1000 microvolts. These were present in nine out of 28 patients (32%) who had both EMG and muscle biopsy. Only one patient (H V 20) had no abnormalities in both. Neurogenic lesions occur in some members of the families and not in others, and are considered part of the disease.