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ORIGINAL COMMUNICATION

Miriam Butz
Manuela C. Koch
Wolfgang Müller-Felber
Richard J. L. F. Lemmers
Silvère M. van der Maarel
Herbert Schreiber

Facioscapulohumeral muscular dystrophy

Phenotype-genotype correlation in patients with borderline D4Z4 repeat numbers

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Dr. Miriam Butz (✉) · M. C. Koch
Institut für Allgemeine Humangenetik
Philipps-Universität Marburg
Bahnhofstr. 7a
35037 Marburg, Germany
Tel.: +49-6421/286-3562
Fax: +49-6421/286-8920
E-Mail: butz@staff.uni-marburg.de

W. Müller-Felber
Friedrich-Baur Institut
Ludwig Maximilians Universität
München, Germany

R. J. L. F. Lemmers · S. M. van der Maarel
Centre for Human and Clinical Genetics
Leiden University Medical Centre
Leiden, The Netherlands

H. Schreiber
Neurologische Klinik
Universität Ulm
Ulm, Germany

Abstract Facioscapulohumeral muscular dystrophy (FSHD) is associated with a decreased number of D4Z4 repeats on chromosome 4q35. Diagnostic difficulties arise from atypical clinical presentations and from an overlap in D4Z4 numbers between controls and FSHD individuals. Thus, a molecular genetic test result with a borderline D4Z4 number has its limitations for the clinician wanting to differentiate between the diagnosis of FSHD and a myopathy presenting with FSHD-like symptoms.

To investigate this problem in more detail we conducted a systematic study of 39 unrelated FSHD patients with borderline D4Z4 repeat numbers and 102 healthy controls. Our aim was threefold: [1] to define the molecular diagnostic cut-off point between FSHD cases and the control population, [2] to describe the myopathic spectrum in patients with borderline D4Z4 repeat numbers and [3] to look for correlations between D4Z4 number and clinical severity.

The results indicate that there is no definite D4Z4 diagnostic cut-off point separating FSHD, FSHD-like myopathies and controls. A broad myopathic spectrum with four phenotypes (typical FSHD, facial-sparing FSHD, FSHD with atypical features, non-FSHD muscle disease) was found in the borderline region. The expected correlation of D4Z4 repeat number and clinical severity was not found. Therefore the molecular test is of limited help to differentiate FSHD from FSHD-like muscle disorders when the D4Z4 number is $n = \geq 8$.

Key words Facioscapulohumeral muscular dystrophy (FSHD) · D4Z4 repeat · facial-sparing FSHD · phenotypical variety in FSHD

Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominantly inherited myopathy with a characteristic pattern of muscular wasting and weakness involving primarily face and shoulder girdle muscles. Later on, foot dorsiflexors, lower abdominal muscles and the pelvic girdle are affected. Extramuscular manifestations such as neurosensory hearing loss and retinal vasculopathy are described. Clinical severity and age of onset may vary widely between and within affected families [9].

A deletion of an integral number of 3.3 kb KpnI repeats (D4Z4) in the subtelomeric region of chromosome 4 (4q35) is associated with the disease [18, 23]. The deletion leads to a reduction of D4Z4 repeats below a critical number on the FSHD-causing allele. The number of remaining D4Z4 repeats on the FSHD allele seems to be directly related to the age at onset and progression of the disease with low repeat numbers causing a more severe phenotype [7].

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Initially, FSHD patients were shown to exhibit on one allele a repeat number smaller than seven units, whereas their non-FSHD allele shows, like control alleles, higher D4Z4 numbers (n = ≥ 8–100) [22, 23]. Later, the critical number of remaining D4Z4 repeats was raised for diagnostic purposes (n = 9–10). At the same time it became evident that in controls the smallest alleles found contained 6–11 D4Z4 repeats [8, 13, 17, 19]. Thus, a remarkable overlap seems to exist between D4Z4 alleles in controls and in FSHD individuals, making a definition of a clear cut-off point difficult. Furthermore, single cases with a typical FSHD phenotype but repeat numbers clearly above the diagnostic threshold (n = ≥ 11) and single patients not completely meeting the diagnostic criteria for FSHD but still within the D4Z4 diagnostic range (n = 8–10) were described [2, 3, 5, 13, 14, 20, 21]. Thus, a molecular genetic test result with a borderline D4Z4 number has its limitations for the clinician wanting to differentiate between the diagnosis of FSHD and a myopathy presenting with FSHD-like symptoms. We therefore initiated a systematic study to characterise suspected FSHD patients with repeat numbers of n = ≥ 8 on a detailed clinical and molecular basis. The aim of the study was threefold: (1) to evaluate whether there is a diagnostic cut-off point between FSHD cases and the control population, (2) to define the myopathic spectrum in patients with borderline D4Z4 repeat numbers, (3) to see whether patients with large residual D4Z4 repeat arrays (= borderline repeat numbers) display a specifically mild phenotype.

Patients

A total of 39 unrelated patients (13 females, 26 males; age at investigation 18–75 years) with a molecular genetic test result of 8–14 D4Z4 repeats were recruited from our records. Of these, 34 patients were personally re-examined by one of the authors (M. B.) and five patients were classified on grounds of the clinical charts. The patient sample comprised 12 familial and 27 sporadic cases.

Clinical examination at patients' homes followed a standardised protocol including a detailed medical history and a neurological examination. The neurological examination included muscle strength testing according to a modified MRC score and a FSHD severity scale [10, 11]. Laboratory, EMG and muscle biopsy data were reviewed.

The study was approved by the ethics committees of the Universities of Marburg and Ulm.

Methods

D4Z4 repeat length determination (± 3.3 kb = ± 1 D4Z4 repeat) was carried out in patients' DNA extracted from blood leucocytes. A differentiation of specific 4q35-*Eco*RI-fragments and unspecific 10q26-*Eco*RI-fragments was performed by double restriction analysis with *Eco*RI and *Bln*I. 4q35-*Eco*RI-fragments persist shortened by 3 kb, whereas 10q26-*Eco*RI-fragments are cleaved into small units and are no longer detectable. Uni-directional gel electrophoresis (0.5 % gels) with a high molecular weight marker as reference for sizing was followed by Southern blot hybridisation with probe p13E-11 (D4F104S1) [1]. D4Z4 repeat numbers were calculated from *Eco*RI-fragment sizes as follows:

$$\text{number of repeats} = \frac{\text{fragment size in kb} - \text{5 kb flanking sequence}}{\text{3.3 kb}} \text{ [16].}$$

Eight and nine D4Z4 repeats (32–35 kb *Eco*RI fragment size) were defined to be the upper diagnostic range for FSHD, 10–11 repeats (38–41 kb) were defined as grey-zone, whereas repeat numbers above 12 (≥ 45 kb) were considered to be beyond the diagnostic range for FSHD (Table 1).

Translocations of 4q35 repeat arrays to chromosome 10 and 10q26 repeat arrays to chromosome 4 are found in approximately 20 % of the normal population. Only deletions of repeats on chromosome 4 cause the FSHD phenotype, no matter whether they are chromosome 4 or chromosome 10 derived [17]. Therefore in patients carrying a FSHD-sized *Bln*I-sensitive fragment, assumed to be of 10q26 origin, a dosage test was performed to rule out translocation events between chromosome 4 and 10 [15]. A further dosage test was performed in those patients, who did not show a typical FSHD phenotype and carried a *Bln*I-resistant fragment to exclude that it is 10q26 derived.

In 102 controls without any obvious sign of muscle disorder (49 male and 53 female volunteers, age 20–50 years) fragment sizes and translocation status were determined using pulsed-field gel electrophoresis (PFGE 0.8 % agarose gel in 0.5xTBE, 23h, 21°C, pulse times 1–16 s, 200Volt).

Results

According to the diagnostic criteria defined by the European Expert Group on FSHD, four phenotypes were

Table 1 Distribution of clinical phenotypes in relation to D4Z4 repeat number

	N	Upper diagnostic range 8–9 D4Z4 repeats (32–35 ± 3 kb)	Grey-zone 10–11 D4Z4 repeats (38–41 ± 3 kb)	Beyond diagnostic range 12–14 D4Z4 repeats (45–51 ± 3 kb)	> 14 D4Z4 repeats (> 51 kb)
FSHD	24/39	17	5	2	–
Facial-sparing FSHD	6/39	5	1	–	–
Atypical FSHD	4/39	3	1	–	–
Non-FSHD	5/39	2	2	1	–
Controls	102	3	4	17	78

found in our patient sample: [1] typical FSHD, [2] facial-sparing FSHD, [3] phenotype with atypical features for FSHD, [4] non-FSHD phenotype [9]. The distribution of the four phenotype categories in relation to D4Z4 ranges for all 39 patients are summarized in Table 1.

Typical FSHD: A total of 24/39 patients undoubtedly fulfilled the diagnostic criteria for FSHD. As expected most patients (n = 17) had D4Z4 repeats within the diagnostic range. Five patients had D4Z4 repeat numbers in the grey-zone, while two patients were clearly beyond the diagnostic range.

Clinical presentation, age at onset and severity did not differ between the three D4Z4 ranges. The same was true for the male to female ratio, creatine kinase (CK) level and family history. In particular, the seven patients with D4Z4 repeats outside the diagnostic range were neither less severely affected nor had they later onset of disease (Table 2).

Facial-sparing FSHD: A FSHD phenotype without facial involvement was found in six patients (Table 3). Five patients had fragments within the diagnostic range, only one patient showed 11 D4Z4 repeats. Interestingly, all facial-sparing FSHD patients were male. Examination of the patients' family members did not reveal any affected relatives except for patient 5. His mother and both sisters were shown to be subclinically affected with mild shoulder weakness; both sisters additionally exhibited facial weakness. According to the diagnostic criteria patient 5 had to be re-classified as typical FSHD, showing the importance of a detailed examination of supposedly unaffected family members.

Atypical FSHD: Four patients presented with atypical features for FSHD. Their features and distribution are shown in detail in Table 3. Three patients fell into the diagnostic range of 8–9 repeats, only one exhibited 11 repeats. The particular clinical features of the latter were characterized by a one-sided atrophy of pectoralis, trapezius and supraspinatus muscles without progression.

Non-FSHD phenotype: After careful clinical re-examination five patients were classified as lacking a FSHD phenotype. Their patterns of muscle involvement and atypical features argued against FSHD. Details are summarized in Table 3. With respect to repeat size, two patients were found within the diagnostic range of 8–9 repeats, whereas two fell with 10 repeats into the grey-zone. Only one patient showed 13 repeats, a repeat number beyond the diagnostic range. The clinical pictures were very heterogeneous, no pattern of clinical presentation seemed to be specifically related to repeat size. This was also true for extramuscular manifestations, laboratory and biopsy findings.

Control population: In our control population we found 3 cases with D4Z4 repeats in the upper diagnostic range of 8–9 D4Z4 repeats, 4 cases in the grey-zone of 10–11 D4Z4 repeats and 17 cases with 12–14 D4Z4 repeats. In total 24/102 presented with repeat numbers from 8–14 (Table 1).

Special findings: Additional short *Bln*I-sensitive (10q26-derived) fragments from 10–48 kb were found in 12/39 patients. All these patients were disomic for 4q35 in the dosage test, thus the *Bln*I-sensitive fragments most likely derive from chromosome 10 and are not associated with the FSHD phenotype. Patients with atypical or non-FSHD phenotype were disomic for 4q35 in the dosage test, thus the *Bln*I-resistant fragment found is most likely chromosome 4 derived.

Discussion

The present study provides evidence that there is no definite D4Z4 diagnostic cut-off point separating FSHD, FSHD-like myopathies and controls. A broad myopathic spectrum with four phenotypes (typical FSHD, facial-sparing FSHD, FSHD with atypical features, non-FSHD muscle disease) is found in the three defined borderline regions: upper diagnostic range, grey-zone and beyond the diagnostic range. There are typical FSHD cases with D4Z4 repeat numbers clearly beyond the diagnostic threshold. No healthy control carried an allele of n ≤ 7 D4Z4 repeats, whereas in accordance with the literature alleles in the upper diagnostic range were found in 3 % of our controls [19]. Therefore, in our experience this

Table 2 Clinical presentation of typical FSHD patients in relation to D4Z4 repeat number

	n	Sex		Age at onset	MRC	Severity[1]			CK (IU/l)	Family history	
		males	females			mild	intermediate	severe		positive	negative
Upper diagnostic range 8–9 D4Z4 repeats	17	9	8	15–40 y	3.2–5	3	9	5	104–286	6	11
Grey-zone 10–11 D4Z4 repeats	5	4	1	13–20 y	3.6–5	2	1	2	120–324	2	3
Beyond diagnostic range 12–14 D4Z4 repeats	2	1	1	16–18 y	4–4.5	–	2	–	not increased	2	–

[1] Severity score according to Ricci et al. 1999: 0.5–1.5 = mild, 2.0–3.0 = intermediate, 3.5–5.0 = severe

Table 3 Summary of clinical presentation in patients not showing typical FSHD

Patient	Classification	D4Z4	Age/sex	Age at onset	Clinical Presentation[1]	Atypical features	CK[2]	EMG[3]	Muscle biopsy	Extramuscular manifestations	Family history
1	facial sparing	8	51/m	11	S* H P D*	no facial involvement	normal	my/neu	n. d.	–	neg
2	facial sparing	9	40/m	28	S* H* P*	no facial involvement	x3	my	n. d.	–	neg
3	facial sparing	9	57/m	19	S* P D*	Facial asymm. w/o paresis	n. d.	n. d.	n. d.	–	neg
4	facial sparing	9	71/m	55	S* H* P D*	no facial involvement	normal	my	mild inflammation	Ventricular arrhythmia	neg
5	facial sparing	9	32/m	25	S* D*	no facial involvement	x5	my	inflammation	–	neg[4]
6	facial sparing	11	20/m	15	S*	Facial asymm. w/o paresis	n. d.	n. d.	n. d.	–	neg
7	atypical	9	55/f	52	F* S* P* D* E*	onset & predominance in left pelvi-femoral muscles	x3	my	n. d.	–	neg
8	atypical	9	38/m	35	S*	isolated atrophy in M. pect. w/o progression	x1.5	neu	n. d.	–	neg
9	atypical	9	69/f	15	F S T P E D	predominance in trunc and pelvic girdle	normal	neu	n. d.	–	possibly mother
10	atypical	11	39/m	31	S*	one-sided atrophy of Mm. pect., trap., suprasp. w/o progression	normal	my	n. d.	–	neg
11	not FSHD	8	54/f	birth	D	bilateral atrophy of Mm. tib. ant. and cong. talipes	x10	my	no pathology	myxomatous heart valves	neg
12	not FSHD	9	75/m	60	N S H Fo P D E	onset in lower extremities; dysarthria, dysphagia	n. d.	n. d.	myopathy	–	grandson
13	not FSHD	10	30/m	23	F*	discrete facial paresis with highly elevated CK	x44	normal	slightly myopathic	–	twin
14	not FSHD	10	47/m		S T P*	improvement under cortisol	x20	n. d.	n. d.	–	neg
15	not FSHD	13	54/m	43	S H T P	onset and predominance in pelvic muscles	x16	my	necrotizing myopathy	–	neg

* asymmetric involvement

[1] F: facial, S: scapular, H: humeral, N: neck, Fo: forearm and hand, T: trunc, P: pelvic, D: foot dorsiflexors, E: foot extensors; [2] x-fold of normal, n. d. = not done; [3] my = myopathic, neu = neurogenic; [4] only after clinical examination mother and two sisters were found to be affected

molecular test is of limited help to differentiate FSHD from FSHD-like muscle disorders when the D4Z4 number is n = ≥8.

It is well accepted that there is a correlation between clinical severity and D4Z4 repeat number: low repeat number stands for early age at onset and severe clinical course, whereas high repeat number means later age at onset and milder course [7]. This correlation between clinical severity and D4Z4 number has recently been supported on an experimental basis [4, 12]. It was shown that a multiprotein complex (YY1, HMGB2, nucleolin) binds to D4Z4 and reduces the expression of the 4q35 genes FRG1, FRG2 and ANT1. In contrast, a D4Z4 deletion results in a pathological overexpression of these genes. It was shown by *in vivo* and *in vitro* studies that the level of overexpression was inversely correlated to the D4Z4 number. This indicates that D4Z4 repeat number is the major factor influencing the extent of the transcriptional misregulation that leads to the FSHD phenotype. Therefore, one might expect predominantly mild affections in FSHD patients with repeat numbers close to normal. This, however, was not the case in our series. No specifically mild phenotype was found in our FSHD patients with borderline repeat numbers. Severe phenotypes were even observed in the grey zone of 10–11 repeats. Thus, our results strongly suggest that other factors than D4Z4 repeat number contribute to the phenotypic severity in the borderline region.

The five patients classified as facial-sparing FSHD showed a pattern of scapulo-humero-peroneal muscle involvement, age at onset and D4Z4 range as seen in typical FSHD. Therefore we agree with other authors that these cases form a subgroup of FSHD instead of a separate disease entity of scapulohumeral myopathy [3, 5].

Our nine atypical and non-FSHD cases were very heterogeneous in phenotype and extramuscular manifestations. We have therefore no compelling evidence to support an expansion of the clinical spectrum of FSHD as seen by other authors [2, 14]. The only exceptions might be patients 8 and 10 with one-sided shoulder weakness (Table 2), who might develop the full FSHD phenotype in the future.

In conclusion, caution is warranted in the diagnostic process and in genetic counselling of cases in the borderline region of 8–14 D4Z4 repeats. Clinicians need to be aware of the limitations of the genetic test in the borderline region. A detailed clinical examination is needed to establish the diagnosis of FSHD in cases with D4Z4 repeat numbers beyond the diagnostic range. On the same grounds, the diagnosis of FSHD should be abandoned in cases with atypical phenotype despite D4Z4 repeat numbers in the upper diagnostic range. In these cases, neither prognosis nor genetic counselling can rely on experiences of cases of FSHD. Keeping in mind that 7/102 of our healthy controls carry one allele with 8–11 D4Z4 repeats, the reductions of D4Z4 number in atypical and non-FSHD patients could represent a coincidental polymorphism. On the other hand it can be hypothesized that with fragment sizes in the borderline region the pathogenic potential of the D4Z4 deletion weakens, making modifying influences necessary for the manifestation of the phenotype. This could give rise to an increasing heterogeneity of clinical symptoms. Further insight might be provided once the relevance of the recently described polymorphic segment distal to D4Z4 is elucidated in these borderline cases [6].

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References

1. Busse K, Köhler J, Stegmann K, Pongratz D, Koch MC, Schreiber H (2000) An inherited 4q35-EcoRI-DNA-fragment of 35 kb in a family with a sporadic case of facioscapulohumeral muscular dystrophy (FSHD). Neuromusc Disord 10:178–181
2. Felice KJ, Moore SA (2001) Unusual clinical presentations in patients harboring the facioscapulohumeral dystrophy 4q35 deletion. Muscle Nerve 24:352–356
3. Felice KJ, North WA, Moore SA, Mathews KD (2000) FSH dystrophy 4q35 deletion in patients presenting with facial-sparing scapular myopathy. Neurology 54:1927–1931
4. Gabellini D, Green MR, Tupler R (2002) Inappropriate gene activation in FSHD. A repressor complex binds a chromosomal repeat deleted in dystrophic muscle. Cell 110:339–348
5. Jardine PE, Upadhyaya M, Maynard J, Harper P, Lunt PW (1994) A scapular onset muscular dystrophy without facial involvement: possible alleism with facioscapulohumeral muscular dystrophy. Neuromusc Disord 4:477–482
6. Lemmers RJ, de Kievit P, Sandkuijl L, Padberg GW, van Ommen GJ, Frants RR, van der Maarel SM (2002) Facioscapulohumeral muscular dystrophy is uniquely associated with one of the two variants of the 4q subtelomere. Nat Genet 32(2):235–236
7. Lunt PW, Jardine PE, Koch MC, et al. (1995) Phenotypic-genotypic correlation will assist genetic counselling in 4q35-facioscapulohumeral muscular dystrophy. Muscle Nerve (suppl. 2): 103–109
8. Orrell RW, Tawil R, Forrester J, Kissel JT, Mendell JR, Figlewicz DA (1999) Definitive molecular diagnosis of facioscapulohumeral dystrophy. Neurology 52:1822–1826
9. Padberg GW, Lunt PW, Koch MC, Fardeau M (1991) Diagnostic criteria for facioscapulohumeral muscular dystrophy. Neuromusc Disord 1(4): 231–234

10. Personius KE, Pandya S, King WM, Tawil R, McDermott MP (1994) Facioscapulohumeral dystrophy natural history study: Standardization of testing procedures and reliability of measurements. Phys Ther 74:253–263
11. Ricci E, Galuzzi G, Deidda G, et al. (1999) Progress in the molecular diagnosis of facioscapulohumeral muscular dystrophy and correlation between the number of KpnI repeats at the 4q35 locus and clinical phenotype. Ann Neurol 45:751–757
12. Tupler R, Perini G, Pellegrino MA, Green MR (1999) Profound misregulation of muscle-specific gene expression in facioscapulohumeral muscular dystrophy. Proc Natl Acad Sci 26: 12650–12654
13. Upadhyaya M, Maynard J, Rogers MT, Lunt PW, Jardine P, Ravine D, Harper PS (1997) Improved molecular diagnosis of facioscapulohumeral muscular dystrophy (FSHD): validation of the differential double digestion. J Med Genet 34:476–479
14. Van der Kooi AJ, Visser MC, Rosenberg N, et al. (2000) Extension of the clinical range of facioscapulohumeral dystrophy: report of six cases. J Neurol Neurosurg Psychiatry 69:114–116
15. Van der Maarel S, Deidda G, Lemmers RJLF et al. (1999) A new dosage test for subtelomeric 4; 10 translocations improves conventional diagnosis of facioscapulohumeral muscular dystrophy (FSHD). J Med Genet 36:823–828
16. Van der Maarel S, Deidda G, Lemmers RJLF, et al. (2000) De novo facioscapulohumeral muscular dystrophy: frequent mosaicism, sex-dependent phenotype, and the role of mitotic transchromosomal repeat interaction between chromosome 4 and 10. Am J Hum Genet 66:26–35
17. Van Deutekom JCT, Bakker E, Lemmers RJLF, et al. (1996) Evidence for subtelomeric exchange of 3.3 kb tandemly repeated units between chromosomes 4q35 and 10q26: implications for genetic counselling and etiology of FSHD1. Hum Mol Genet 5: 1997–2003
18. Van Deutekom JCT, Wijmenga C, van Tienhoven EAE, et al. (1993) FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit. Hum Mol Genet 2:2037–2042
19. Van Overveld PGM, Lemmers RJFL, Deidda G, et al. (2000) Interchromosomal repeat array interactions between chromosomes 4 and 10: a model for subtelomeric plasticity. Hum Mol Genet 9:2879–2884
20. Vielhaber S, Jakubiczka S, Schröder JM, et al. (2002) Facioscapulohumeral muscular dystrophy with EcoRI/BlnI fragment size of more than 32 kb. Muscle Nerve 25:540–548
21. Vitelli F, Villanova M, Malandrini A, et al. (1999) Inheritance of a 38-kb fragment in apparently sporadic facioscapulohumeral muscular dystrophy. Muscle Nerve 22:1437–1441
22. Weiffenbach B, Dubois J, Storvick, D, et al. (1993) Mapping the facioscapulohumeral muscular dystrophy gene is complicated by chromosome 4q35 recombination events. Nat Genet 4: 165–169
23. Wijmenga C, Hewitt JE, Sandkuijl LA, et al. (1992) Chromosome 4q DNA rearrangements associated with facioscapulohumeral muscular dystrophy. Nat Genet 2:26–30