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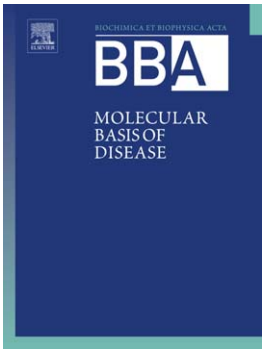

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PII: S0925-4439(06)00100-1
DOI: doi:10.1016/j.bbadis.2006.05.009
Reference: BBADIS 62586

To appear in: *BBA - Molecular Basis of Disease*

Received date: 3 May 2006
Revised date: 30 May 2006
Accepted date: 30 May 2006

Cite this article as: Silvère M. van der Maarel, Rune R. Frants, George W. Padberg, Facioscapulohumeral muscular dystrophy, *BBA - Molecular Basis of Disease* (2006), doi:10.1016/j.bbadis.2006.05.009

Facioscapulohumeral muscular dystrophy

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keywords: FSHD, muscular dystrophy, review, D4Z4, therapy, epigenetic

Abstract

Facioscapulohumeral muscular dystrophy (FSHD) is caused by a cascade of epigenetic events following contraction of the polymorphic macrosatellite repeat D4Z4 in the subtelomere of chromosome 4q. Currently, the central issue is whether immediate downstream effects are local (i.e. at chromosome 4q) or global (genome-wide) and there is evidence for both scenarios. Currently, there is no therapy for FSHD, mostly because of our lack of understanding of the primary pathogenic process in FSHD muscle. Clinical trials based on suppression of inflammatory reactions or increasing muscle mass by drugs or training have been disappointing. A recent, probably the first evidence-based pilot trial to revert epigenetic changes did also not provide grounds for a larger clinical study. Clearly, better disease models need to be developed to identify and test novel intervention strategies to eventually improve the quality of life for patients with FSHD.

Outline of this review

Autosomal dominant facioscapulohumeral muscular dystrophy (FSHD) is a common myopathy with a unique epigenetic etiology. With almost complete linkage to the distal end of chromosome 4q (4q35), this disorder is not caused by structural mutations within the disease gene as commonly seen in monogenetic traits, but rather involves a complex cascade of epigenetic events following contraction of a subtelomeric macrosatellite repeat. This review aims to discuss recent advances in our understanding of FSHD pathology viewed against its clinical heterogeneity. We will also discuss recent clinical trials and speculate on novel directions for therapy.

Clinical features

Patients usually present with symptoms related to weakness of the scapula-fixators; rarely do they report onset of the disease in the facial, foot-extensor or pelvic girdle muscles. Shoulder girdle weakness characteristically is visible as an asymmetrical scapula alata and pectoralis muscle atrophy. An initially fairly normal deltoid muscle contributes to the distinctive high risk of the scapula on elevation of the arms [1,2]. Another, very typical feature is the asymmetrical facial weakness. If subtle facial weakness is taken into account more than 90 % of patients reveal facial muscle involvement. In large families 30 % of gene carriers have no complaints and often do not know they are affected. A number of patients do not progress beyond shoulder muscle involvement and in large families approximately half the patients progress to pelvic girdle weakness, usually after extension of the weakness to the abdominal, foot-extensor and upper-arm muscles. Landouzy and Dejerine described the extreme hyperlordosis as a characteristic end-stage of this course [3].

A patient usually recognizes the disease in his teens but an extreme variation in onset is reported ranging from early infancy to late fifties, even within a family with all affected members carrying the same genetic lesion. When the disease progresses to the lower limbs again the rate of progression is quite variable [2]. Occasionally long periods of standstill have been reported. A mild gender effect has been noted by several authors; women tend to be more often asymptomatic, have a slightly later onset and a somewhat milder course of the disease [4]. Muscle pain and fatigue are frequently reported; dysphagia is rare, as is respiratory insufficiency [5]. Occasionally cardiac conduction defects have been observed, although most authors claim no cardiac involvement. Extra-ocular and masticatory weakness are no part of FSHD and point to other diagnoses. Lingual muscle involvement, probably not progressive, has been mentioned in early onset FSHD [6].

Contractures are rare with exception of ankle contractures. A subclinical high tone hearing loss and a subclinical retinal vasculopathy have been described as part of the disease [7,8]. Rarely, and almost exclusively in early onset disease, they become symptomatic.

Early, i.e. infantile, onset has in the past been thought of as an independent entity but has turned out to be the more severe end of the clinical spectrum with usually small residual D4Z4 repeat sizes (see genetic defect), while the other end of the spectrum reveals often minimally to mildly affected patients with probably more frequently non-penetrant gene carriers in these families.

The characteristics of FSHD often lie in subtle signs when a physician is confronted with a patient with a facio-scapulo-humeral syndrome. But the rarity of the disorders in the differential diagnosis make that FSHD should be considered first [2]. Genetic diagnostics is recommended as the first step and, only when FSHD has been ruled out, family examination, EMG and muscle biopsy are indicated.

Genetic defect

Linkage for FSHD was established in the early nineties by a genome-wide microsatellite scan in a few Dutch families with autosomal dominant FSHD. FSHD was found to link to 4q35, which was confirmed in other families worldwide [9-13]. By means of positional cloning the genetic defect in FSHD was identified as a reduction of the size of an *Eco*RI fragment on genomic DNA when hybridised with probe p13E-11. This probe was derived from a cosmid clone mapping to 4q35 that was isolated in a screen for homeobox-like sequences. In familial and isolated FSHD cases this large and polymorphic *Eco*RI fragment was reduced below the size of 28 kb [14,15]. Not much later, the genetic defect in FSHD was established as a partial deletion of an integral number of repeat *Kpn*I fragments, designated D4Z4 [16]. Screening of large series of control individuals and FSHD patients showed that the D4Z4 repeat normally varies between 11-100 units, giving rise to *Eco*RI fragments of 40 > 300 kb, while >95% of FSHD patients carried one allele of 1-10 units (*Eco*RI fragments of 10–38 kb) [17]. FSHD patients with one allele completely devoid of D4Z4 units have never been reported and monosomy 4q does not cause FSHD predicting a critical role for D4Z4 in FSHD pathogenesis [18]. From now on, DNA diagnosis for FSHD became available. Over recent years, it has become apparent that there are many complications to this relative straightforward diagnosis of FSHD, which we will briefly discuss below.

First, it was shown that an almost identical and equally polymorphic repeat resides in the subtelomere of chromosome 10q as a result of an ancient duplication [19,20]. Repeat contractions on chromosome 10q are non-pathogenic [21,22]. Second, apparent unconstrained exchanges between repeat units on both chromosomes can be encountered in the population and although generally the precise allele constitution for all chromosomes 4 and 10 can be precisely defined, even for the most complicated repeat exchanges, it needs sophisticated

follow-up experiments often not available in diagnostic centres. Repeat exchanges between chromosomes 4 and 10 can be encountered in approximately 10% of the population, but despite this dynamic behaviour, FSHD is uniquely linked to chromosome 4 and contractions of translocated 4-type repeat units on chromosome 10 have never been observed in FSHD [23-25].

In addition to a contraction of D4Z4, at least one yet to be identified cis-element seems to be necessary to develop FSHD. A biallelic variation was discovered for the 4q subtelomere [26]. Both variants, 4qA and 4qB, are almost equally common in the population, but FSHD alleles are always of the 4qA-type, except for a few very rare cases in which the disease allele failed to hybridise to the 4qA and 4qB probe [27,28]. While both variants show an equal variation is size and an equal somatic instability, it was concluded that additional elements on 4qA were necessary to cause disease or 4qB chromosomes carry elements that prevent FSHD. Indeed, several families have now been reported in which FSHD-sized 4qB alleles segregate in healthy individuals [29,30]. Both variants differ by few insertion deletion events, the most prominent being a large block of beta satellite immediately distal to D4Z4 on 4qA alleles [26]. Although initially the elements that distinguish 4qA from 4qB were assigned to the region distal to D4Z4, a region of approximately 60 kb ending in the telomere repeat, more recent studies show that these sequences distal to D4Z4 are in strong linkage disequilibrium with sequences immediately proximal to D4Z4. These studies have also indicated that 4qA and 4qB alleles infrequently exchange material in the regions proximal and distal to D4Z4 feeding the assumption that both alleles are functionally different [31].

In a small percentage of FSHD cases, the disease allele can be easily overlooked as the deletion in these patients extends in proximal direction and includes the probe region p13E-11 [23,32]. While solutions have been provided to overcome this problem in DNA diagnosis,

these patients may be very instrumental in further defining the minimal requirement to develop disease.

In summary, in >95% of FSHD cases, a contraction of D4Z4 below the threshold of 11 units on a 4qA allele is associated with disease. For the remainder of patients no locus has been identified although recently some evidence for linkage to chromosome 15 was presented in one family [33,34]. Some candidate genes, most notably those encoding for components of the D4Z4 repressor complex (see epigenetic disease mechanism) and *MYOD1*, have been excluded as candidate genes [35]. However, recently it was shown that some of these cases share an epigenetic phenomenon with 4qter-linked FSHD and therefore in these cases, the disease mechanism also acts through chromosome 4 (see epigenetic disease mechanism). Other cases have wrongly been attributed to genetic heterogeneity as they represent cases with proximal extended deletions preventing identification of the contracted allele by standard DNA diagnosis. Whether or not true non-4q-linked FSHD cases exist remains to be established.

Epigenetic disease mechanism

After the identification of the primary genetic lesion in FSHD, research focused on the gene content within D4Z4 assuming that contraction of D4Z4 directly impaired the structure or function of a gene contained within D4Z4. Each D4Z4 units has sequence features characteristic for a CpG island with a GpC:CpG ratio of 0.8. It also contains several repeat sequences suggestive for a heterochromatic structure. Moreover, a putative gene was identified within each unit designated *DUX4* (for double homeobox gene 4) [36-39]. Despite many efforts, no conclusive evidence has been presented thus far that this gene is actively transcribed *in vivo* and encodes for a genuine homeobox protein. However, at present it cannot be excluded that in specific spatiotemporal windows, *DUX4* is expressed.

Approximately 40 kb proximal to D4Z4, one inverted and truncated copy of D4Z4 is located [40]. This inverted copy defines the proximal boundary of the region of homology between chromosomes 4 and 10 and also contains a putative double homeobox gene *DUX4C*, which is absent on chromosome 10. Like for *DUX4*, no expression is documented for *DUX4C* [41].

Therefore, focus shifted to understanding the chromatin structure of D4Z4 -and possibly, a change thereof in FSHD patients- and to the identification of candidate genes in the region immediately adjacent to the D4Z4 repeat assuming that the D4Z4 repeat can influence the transcriptional control of 4q35 genes. A DNA-binding complex, consisting of HMG2B, YY1 and nucleolin, was identified that binds to D4Z4. It was proposed that this complex acts as a local repressor and that with insufficient binding due to the loss of sufficient binding sites, local genes became transcriptionally upregulated. Consistent with this view was their observation that in FSHD muscle three genes, *FRG1*, *FRG2*, and *ANT1*, were transcriptionally upregulated. This upregulation was distance-dependent with the gene closest to D4Z4 being most significantly upregulated and D4Z4 repeat size-dependent: patients with smaller residual repeat sizes showed greater upregulation than patients with longer residual repeat sizes. Moreover, in cell cultures devoid of members of the repressor complex showed transcriptional upregulation of *FRG2* [42].

However, several independent follow-up studies using different quantitative expression analysis techniques failed to reproduce the transcriptional upregulation of above-mentioned genes in FSHD skeletal muscle [43,44]. Expression levels varied from unchanged to upregulated or even downregulated compared to normal muscle. This issue is still largely unresolved but may partly be explained by the different technological approaches and variation in source of RNA (muscle pathology, duration of disease, etc.). At protein level, only limited data are available as discussed below for the individual candidate genes.

Studies of DNA methylation showed that D4Z4 at disease alleles is significantly hypomethylated. Employing two methylation-sensitive restriction enzymes, normal alleles showed average methylation levels of approximately 50% while in FSHD alleles, methylation levels were on average reduced by a factor 2. Interestingly, some phenotypic FSHD patients without contraction of D4Z4, also showed pronounced hypomethylation of D4Z4. In contrast to 4q-linked FSHD, where the hypomethylation was restricted to the contracted (disease) allele, in phenotypic FSHD, both chromosomes 4 were hypomethylated at D4Z4. Non-penetrant gene carriers of 4q-linked FSHD families also showed hypomethylation at D4Z4. Collectively, these data show that hypomethylation of D4Z4 is necessary but not sufficient to cause FSHD [45].

Although overall FSHD alleles are hypomethylated at D4Z4, individual methylation levels are very diverse. To explain inter- and intrafamilial variation in disease severity, it was postulated that variation in residual D4Z4 methylation may influence the natural course of disease. Although in a small set of patients no direct linear relationship could be established between D4Z4 hypomethylation and disease severity, it is interesting that the smallest FSHD repeats (which are commonly associated with the severest phenotypes) showed the lowest levels of D4Z4 methylation. Clearly, more comprehensive methylation assays need to be developed to address the issue of D4Z4 methylation-dependent severity [46].

Other studies of chromatin structure of 4qter show that the FSHD region should be considered as unexpressed euchromatin, rather than heterochromatin. Gene expression studies and histone H4 acetylation levels in regions immediately adjacent to D4Z4 and in more proximal direction did not show evidence for a change in chromatin structure emanating from D4Z4 between cell lines from controls and FSHD patients. Also other markers for heterochromatin, including co-localization studies of 4qter with DAPI bright foci or enrichment of 4qter for

histone H3 trimethylated at lysine 9 and heterochromatin protein 1a, were consistent with D4Z4 being unexpressed euchromatin [43,47].

A few genes have been considered as good candidate genes for FSHD based on their localization and / or function. The gene that was first investigated for its potential role in FSHD is *ANT1* gene coding for the adenine nucleotide translocator. This gene is located at minimal 3,5 Mb distance of D4Z4 and encodes for a protein that facilitates the export of ATP over the mitochondrial membrane [48-50]. Its high expression in skeletal and heart muscle and its involvement in several neuromuscular disorders made it an attractive candidate gene but expression studies to determine whether ANT1 levels are increased in FSHD muscle remain controversial. Recently, evidence was presented that ANT1 protein levels were increased in asymptomatic and symptomatic muscle [51], but additional studies are necessary to explore the involvement of ANT1 in FSHD as transgenic mice muscle-specifically overexpressing *ANT1* do not seem to develop muscular dystrophy [52].

Another gene which involvement was tested in FSHD is *PDLIM3*. This gene encodes for the muscle-specific actinin-associated LIM protein (also known as *ALP*) but Westernblot analysis and immunohistochemistry did not provide evidence for differential expression of the PDLIM3 protein [53,54]. PDLIM3 knockout mice show no evidence for muscular dystrophy but present with right ventricular chamber dilatation, making this protein an unlikely candidate for FSHD [55].

The gene closest to D4Z4 is *FRG2*. The *FRG2* gene, at 37kb distance from D4Z4 encodes a putative nuclear protein of unknown function [56]. Although specifically upregulated in differentiating muscle cell cultures of FSHD patients, its absence in some patients with proximally extended deletions, makes this gene a less attractive candidate [32]. Also, mice overexpressing *FRG2* do not present with muscular dystrophy [52].

The last candidate gene investigated at 120kb distance from the D4Z4 repeat is *FRG1*. This gene is highly conserved in vertebrates and non-vertebrates and encodes a nuclear protein. All evidence suggests that FRG1 is a spliceosomal protein, but the exact function remains to be elucidated [57-59]. Interestingly, the subtelomeric localization of *FRG1* is hominoid-specific. Due to an ancient translocation in all species but hominoids *FRG1* is located in a gene-rich domain flanked by *PCM1* and *ASAH1* [60]. Since its translocation to the 4q subtelomere, several duplication events have occurred yielding almost identical copies of *FRG1* at many chromosomes, some of which also seem to be actively transcribed [58,61].

Expression studies of *FRG1* in FSHD muscle are controversial. Various studies employing semi-quantitative radioactive RT-PCR, quantitative RT-PCR, expression arrays and even allele-specific RT-PCR on affected muscle of FSHD patients yielded evidence varying from no change in expression levels to 25-fold upregulation or even 5-fold downregulation [42-44,58]. Clearly, the different techniques used and the natural variation in the source of RNA (site of biopsy, pathology, disease duration, severity) has contributed to this controversy. However, with the recent observations in the transgenic *FRG1* mice (see below), solving the issue of *FRG1* expression in FSHD muscle has even become more important.

Mice that overexpress *FRG1* in skeletal muscle develop muscular dystrophy with a severity proportional to the level of overexpression. Three transgenic lines were generated overexpressing *FRG1* 10-, 25- and 45-fold, respectively, compared to the endogenous *Frg1* levels. While the mice with lowest overexpression did not show significant pathology, mice with intermediate or high FRG1 levels showed increasing pathology. Interestingly, missplicing of specific mRNAs was observed in skeletal muscle of these mice further corroborating on the observation that FRG1 is a spliceosomal protein. Missplicing was also observed in C2C12 cells stably expressing HA-tagged FRG1 and in muscle cell cultures of FSHD patients [52]. However, we did not find evidence for missplicing in RNA from affected

muscle of FSHD patients (unpublished data). Missplicing of muscle-specific mRNAs has been reported earlier in myotonic dystrophy (DM) raising the intriguing possibility of a commonality between FSHD and DM. However, until the controversy of *FRG1* upregulation is truly solved, it is too early to assign *FRG1* as the causative gene for FSHD.

Studies of nuclear organization

An entirely different and equally exciting finding relates to commonalities that FSHD may share with the nuclear envelope dystrophies. Chromosomes occupy distinct territories in the mammalian nucleus. These chromosome territories are often a reflection of their gene density, transcriptional activity, replication timing, and chromosome size [62,63]. Unlike all other chromosome ends studied, including the highly homologous 10qter, 4qter is preferentially localized in the outer nuclear rim, independent of cell type and chromosome territory effects. Not D4Z4 itself, but sequences proximal to D4Z4 seem to be necessary and sufficient for this perinuclear localization [64,65]. This may explain the different nuclear localization of 10qter as the homology between 4qter and 10qter only extends 40kb proximal to D4Z4. Although no difference was observed in localization of normal and FSHD chromosomes, the perinuclear localization of 4qter is largely lost in fibroblasts lacking lamin A/C [64]. The nuclear envelope dystrophies, including X-linked and autosomal dominant Emery-Dreifuss muscular dystrophies (EDMD) are caused by mutations in emerin and lamin A/C, respectively, thus raising the intriguing possibility that FSHD is related to these nuclear envelope muscular dystrophies [66]. Indeed, recent expression studies showed that the transcriptome fingerprints of EDMD and FSHD are highly similar [67].

Clinical trials

In 1997 the natural history of FSHD was described based on a careful systematic follow-up of 81 patients. This study also calculated that a two armed clinical trial with a power of 80 % to detect arrest of progression after 1 year would require 160 patients in each arm [68].

This observation led to the strategy of pilot-studies in a small number of patients aiming for large effects before deciding on large trials, and it pushed for a search for biomarkers as surrogates to follow before planning large-scale studies.

First, prednison 1.5 mg/kg/day was tested in an open label study in 8 patients, because of the frequent observation of inflammatory infiltrates in muscle biopsies. The hope was for a similar effect as in Duchenne muscular dystrophy. However, after 3 months no significant changes in muscle mass and muscle strength were noted [69].

Subsequently albuterol was studied because of its known effect on muscle protein-metabolism and contractility, and experiences in sports medicine. A slightly successful 3-month open label pilot was followed by a randomized placebo-controlled trial in 90 patients testing two doses (16 and 32 mg/day) during one year. After 52 weeks mean strength in the treatment and in the placebo group was the same. As the treatment group showed initially a small gain in strength (similar to the pilot condition) it was suggested that the anabolic effect wears off over time [70].

A similar study testing albuterol 16 mg/day for 6 months was carried out in the Netherlands. The results showed significant improvement of isometric muscle strength in 7 out of 12 muscle groups and increased muscle volume but there was no positive effect on pain, experienced fatigue, functional status (Sickness Inpact Profile) and on psychological distress [71].

Creatine plays an important role in the energy metabolism of skeletal muscle food supplement as patients with muscular dystrophies were reported to have lower endogenous muscle stores.

A randomized double blind, cross-over study in 32 patients (12 FSHD) showed a significant positive effect on muscle strength and activity scores for the group as a whole [72]; analysis of the FSHD patients only revealed no positive effects. However, this study was deemed too small and too short for firm conclusions [73].

Based on personal observations Lefkowitz and Lefkowitz argued for a role of calcium-entry-blockers in the treatment of FSHD [74]. An open label pilot-study of diltiazem 30 mg TID was carried out in 20 patients. Muscle strength and muscle mass was not changed significantly after 6 months. No future studies were suggested [75].

Because we recently demonstrated that the shortened D4Z4 repeat in FSHD is hypomethylated we designed an open-label pilot to study the effects of folic acid (5 mg/day) and methionine (1 gm TID) on D4Z4 methylation after 12 weeks in 9 FSHD patients and in 6 healthy controls. No significant changes in methylation level of D4Z4 were found in patients or controls(v.d. Kooi unpublished).

At present a clinical trial is under way testing recombinant myostatin-neutralizing antibody treatment in a number of Becker, limb-girdle and FSH muscular dystrophy patients. As myostatin negatively regulates muscle mass, blocking myostatin might reverse a muscle wasting condition.

The effects of training of muscle strength in FSHD have been under discussion for years. Only recently training was evaluated in a prospective randomized controlled, single blinded study [71]. Training for 6 months was followed by a double-blinded addition of albuterol 16 mg/day. Training included dynamic and isometric excercises. Maximum voluntary isometric strength was unchanged at 52 weeks in the ellow flexors but decreased -though not significantly- in the foot dorsiflexors. Dynamic strength tests of 52 weeks showed a significant positive effect in the elbow flexors only. The differences in response between the

elbow flexors and foot dorsiflexors remained unclear. If was concluded that training does not inflict harm to FSHD muscles.

Similarly, a 12 weeks, aerobic training programme on a cycle ergometer at a heart rate corresponding to a work at 65 % of VO2-max in 8 FSHD patients showed no adverse affects on quadriceps histology. Maximal oxygen uptake and workload improved significantly and most patients reported subjectively improved strength and activity [76]. Fatigue did not improve, suggesting that fatigue is different from deconditioning. As most patients in this study were mildly affected further training studies in more severely affected patients appear desirable.

Possibilities for therapy

All trials thus far for FSHD have been rather disappointing. If any positive effect was observed, generally it did not persist or was too subtle to significantly improve the quality of life for the patients. At present, molecular studies are not sufficiently advanced to suggest new therapeutic handles on the primary or early pathogenic mechanisms. Clinical trials so far have addressed end of cascade features and fairly advanced stages of muscle pathology. A combination of presently known therapies is not likely to yield a significant improvement in muscle performance. The one combination trial of training and albuterol did not show an additive effect [71].

Nevertheless, our observations in mosaic patients seem to indicate that we may only need to correct the pathogenic mechanism in a proportion of cells to slow down or halt disease progression. Individuals mosaic for the disease allele due to a mitotic contraction of D4Z4 only have a proportion of affected cells and generally present with a much milder, or even (almost) asymptomatic, phenotype [77]. Most notably in mosaic females, the disease presentation is often so mild that it is only recognized after the diagnosis of an affected child

that inherited the affected allele. Although there seems to be a correlation between the severity and a combination of proportion of affected cells in PBL and residual repeat size, only muscle of a single mosaic female was available to demonstrate that muscle and blood show equal proportion of affected cells [30]. Clearly, additional mosaic cases need to be studied to compare mosaic cell proportions in blood and muscle and to define the minimal proportion of normal nuclei in FSHD muscle to exert beneficial effects.

Interestingly, it was recently demonstrated that muscle cell cultures derived from unaffected muscle (vastus lateralis) of FSHD patients were morphologically and behaviourally indistinguishable from healthy muscle cell cultures. These cells had normal morphological appearance as assessed by light microscopical analysis and were able to differentiate to myotubes with similar efficiency as wild type muscle cells. Moreover, when injected in immunodeficient mice, these cells were able to participate in muscle regeneration processes [78]. These data are in marked contrast to earlier studies of FSHD muscle cell cultures derived from affected muscle. These cells displayed a necrotic phenotype with enhanced susceptibility to oxidative stress [79]. While these studies indicate that there may be a functional difference between muscle cells from affected and unaffected muscle, they also demonstrate the possibility of autologous myoblast transplantation. However, without having corrected for the pathogenic mechanism in these cells, it is difficult to envisage a lasting beneficial effect of autologous myoblast transfer in affected muscle of FSHD patients. Therefore, how can we correct this mechanism?

Owing to the complex genetic rearrangement in FSHD with many copies of homologous D4Z4 sequences in the genome, it is unlikely that a gene therapy approach to insert additional D4Z4 units at the disease locus is a feasible option. Therefore, other therapeutic options that directly affect immediate downstream mechanisms of the D4Z4 contraction should be considered.

As disease alleles are variably hypomethylated at D4Z4, we explored the possibility of remethylating D4Z4. Several *in vivo* folate depletion and repletion studies in humans have shown positive effects on total genomic DNA methylation and expression of methylation-regulated genes in PBL and several body tissues [80,81]. Other studies indicate that the effect of folate status on DNA methylation is more complex [82]. As mentioned, in our small open trial, while in most subjects total genomic methylation levels increased as expected, we were unable to identify significant remethylation of D4Z4 (Van der Kooi, unpublished).

Other approaches to correct the pathogenic mechanism in FSHD need to come from our steadily increasing understanding of the primary defect in FSHD and its immediate consequences. The central issue that is emerging seems to be whether these immediate effects are local, global, or both. Based on recent development, arguments can be made favouring immediate local and global pathogenic consequences.

The loss of the D4Z4 repressor complex at the disease allele may not only have a local effect on transcriptional repression of 4q35 genes, but it is conceivable that it may also have genome wide consequences. Clearly, the issue of transcriptional deregulation of 4q35 genes in FSHD muscle is still not resolved and a global effect of YY unbalance may come from a recent study in which it was shown that YY1 binds to Enhancer of Zeste 2 (Ezh2), a member of the Polycomb group proteins, which is developmentally regulated in the myotome compartment of mouse somites [83]. Ezh2 has histone lysine methyltransferase activity and interacts with histone deacetylase HDAC1 to exert transcriptional repression. In proliferating primary myoblasts, the YY1-Ezh2 complex is located, in combination with HDAC1, on regulatory regions of transcriptionally inactive muscle-specific genes. Ttranscriptional activation of these genes during myoblast differentiation coincides with loss of Ezh2-YY1 at their regulatory elements and the recruitment of the transcription factor MyoD. As for transcriptional regulators generally a strict stoichiometry is required, these observations raise the intriguing

possibility that a local unbalance in YY1 binding to D4Z4 may have genome-wide effects in FSHD.

The hypothesis that immediate downstream effects from the D4Z4 contraction are not limited to 4q35 is supported by transcriptome and nuclear localization studies. Transcriptome analysis has shown global changes in gene expression in FSHD muscle and also provides evidence for a defect in specific stages of muscle differentiation. Amongst the genes differentially expressed in FSHD muscle, many of them are immediate targets of MyoD [44]. A more recent cross-sectional transcriptome study showed that the transcriptional profile of FSHD co-segregated with that of the nuclear lamina dystrophies, caused by mutations in emerin and lamin A/C [67]. This substantiates the earlier proposal that, based on the consistent localization of 4qter in the outer rim of the nucleus, and its dissociation from the nuclear periphery in lamin A/C null fibroblasts, FSHD should be considered as a nuclear envelope disease [64]. Locally, the intriguing observation that muscle specific overexpression of *FRG1* in transgenic mice leads to muscular dystrophy makes this protein a prime candidate to be further studied in relation to FSHD.

According to Dubowitz [84], the rationale for clinical intervention studies should be based on one or more of the following premises: (1) theoretical grounds for the potential value of a drug in relation to a hypothesis on the pathogenesis of FSHD, (2) trials of therapeutic agents in animal models of the disease, (3) extrapolation from the use of a drug in similar neuromuscular or degenerative disorders, (4) extrapolation from the use of an agent used to enhance muscle performance and recovery or to increase muscle mass in for example sports and veterinary medicine or (postsurgery) rehabilitation, (5) observations in relation to experimental situations such as muscle tissue culture, and (6) serendipity.

With the recent developments in FSHD, we expect trials based on the hypothesis of FSHD pathogenesis to emerge in the years to come. Probably, our folic acid trial was the first of this

kind. However, with the lack of a widely recognized faithful animal model for FSHD, the development of new therapeutic agents for FSHD is strongly hampered. For premises 3 and 4, all trials have been relatively disappointing with only the myostatin-neutralizing antibody MYO-029 trial still ongoing. Even the observations in primary muscle cell cultures of FSHD patients (5) are not consistent and need further attention. Ideally, a faithful uniform and (conditionally) immortal cell model needs to be developed to explore potential therapeutic strategies. Unfortunately, the last premise, serendipity, has not brought FSHD any luck.

Acknowledgements

FSHD research is supported by grants from the Netherlands Organization for Scientific Research, the Prinses Beatrix Fonds, the Stichting Spieren voor Spieren, the Muscular Dystrophy Association (USA), the Dutch FSHD Foundation, the National Institutes of Health (NIH-NIAMS; R21 AR4 8327-01), the Shaw family and FSH Society, Inc.

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Figure legends

Figure 1, complex (epi)genetic cascade causing FSHD. FSHD is caused by contraction of the polymorphic D4Z4 repeat (triangles) in the subtelomeric domain of chromosom 4q to a residual size of 1-10 units. This contraction is paralleled by DNA hypomethylation (open circle: unmethylated DNA, closed circle: methylated DNA) and other chromatin changes including a possible partial loss of the D4Z4 repressor complex. To exert a pathogenic effect, D4Z4 contractions need to occur on one of the two common variants of 4qter, the 4qA allele (hatched box). D4Z4 contractions on the 4qB allele are non-pathogenic and it is currently unclear which functional domains are responsible for this difference. In a small proportion of patients, the so-called phenotypic FSHD patients, hypomehthylation of D4Z4 is also observed unrelated to contraction. These changes in chromatin structure are hypothesized to cause a cascade of downstream effects including local and global deregulation of transcription. Several genes within the vicinity of D4Z4 have been investigated for their role in the development of FSHD. Most notably, these include FRG2 (at 37Kb distance), FRG1 (at 120Mb distance) and ANT1 (at approximately 3Mb distance).