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Genetic and Epigenetic Contributors to FSHD

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Abstract

Facioscapulohumeral dystrophy (FSHD) is an autosomal dominant muscle disorder characterized by distinct chromatin changes including DNA hypomethylation of the D4Z4 macrosatellite repeat array on a disease-permissive 4qA allele and aberrant expression of the D4Z4-embedded *DUX4* retrogene in skeletal muscle. Insufficient epigenetic repression of the D4Z4 repeat is the result of at least two different genetic mechanisms leading to two forms of disease, FSHD1 and FSHD2. In the case of FSHD1, a contraction of the D4Z4 repeat array is disease causing whereas FSHD2 is most often caused by mutations in the structural maintenance of chromosomes hinge domain 1 (*SMCHD1*) gene. Recent studies indicate that a combination of genetic and epigenetic factors that act on the D4Z4 repeat array determine the probability of *DUX4* expression in skeletal muscle and disease penetrance and progression.

Introduction

Facioscapulohumeral dystrophy (FSHD) is a common muscular dystrophy characterized by initial weakness of the facial, shoulder and upper arm muscles but progressing to affect almost all skeletal muscles [1]. In families with the most frequent genetic cause of the disease (FSHD1), a contraction of the polymorphic D4Z4 macrosatellite repeat array on a specific FSHD-permissive haplotype of chromosome 4q, the disease segregates as a dominant trait [2–4]. In a small proportion of cases, the disease is caused by mutations in *SMCHD1*, a member of the condensin/cohesin family of chromatin compaction complexes that binds to the D4Z4 repeat array [5]. In these FSHD2 families, the disease shows a more complex digenic inheritance because the mutation of *SMCHD1* on chromosome 18 segregates independently from the FSHD-permissive chromosome 4q haplotype [5]. In both forms of the disease, the mutations are associated with a partial relaxation of the D4Z4 chromatin structure and a failure to repress *DUX4*, a transcription factor normally expressed at high levels in germ cells of human testes, encoded by the D4Z4 unit in skeletal muscle [6–10].

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The D4Z4 macrosatellite repeat array

The D4Z4 macrosatellite repeat is a polymorphic array consisting of 3.3kb D4Z4 units and is located in the subtelomere of chromosome 4q, at approximately 40kb distance to the telomere repeat (Fig. 1). Due to a duplication event, a highly similar and equally polymorphic repeat array localizes to the subtelomere of chromosome 10q [11]. In the non-FSHD population, the size of the D4Z4 repeat array varies between 1–100 units on both chromosomes, except for FSHD permissive 4qA alleles (see below) where it varies between 11–100 units. At sizes >10 units it adopts a repressed chromatin structure in somatic cells as evidenced by high levels of CpG methylation and the presence of repressive histone modifications [7, 10]. Each D4Z4 unit contains repetitive sequences typically found in heterochromatic DNA, but the most prominent feature of the D4Z4 unit is the presence of an open reading frame for a retrogene called *DUX4* [12, 13]. *DUX4* is believed to have resulted from retrotransposition of *DUXC*, a gene present in many mammals but lost in the primate lineage [14]. The *DUX4* retrogene encodes a double homeobox transcription factor, which is normally expressed in the testis (Fig. 1), and epigenetically silenced in somatic cells [8].

D4Z4 repeat array contraction in FSHD1

In patients diagnosed with FSHD1 the D4Z4 repeat array is contracted to 1–10 copies (Fig. 1). In approximately half of the *de novo* cases (representing 10–30% of FSHD1), the contractions occurs somatically, most likely by an intrachromosomal gene conversion mechanism with or without cross over, and leading to somatic mosaicism for the disease allele [15]. D4Z4 contractions to a size between 1–10 units are associated with partial relaxation of the D4Z4 chromatin structure in somatic cells (mostly determined by D4Z4 hypomethylation) and transcriptional derepression of the *DUX4* retrogene in skeletal muscle [6–10, 16]. Population studies have indicated that sizes between 8–11 units represent a grey zone where, depending on the epigenetic state of the D4Z4 repeat array, disease penetrance is incomplete [17–19]. Moreover, individuals with monosomy 4qter and thus a single intact D4Z4 repeat array do not exhibit FSHD [20].

A critical role for DUX4 in FSHD pathophysiology

The *DUX4* retrogene in the D4Z4 repeat unit lacks its own polyadenylation signal but in the testis it makes use of a germline specific polyadenylation signal far distal to the D4Z4 repeat array (Fig1) [8]. In FSHD, where *DUX4* becomes aberrantly expressed in somatic cells, it makes use of a polyadenylation signal that is polymorphic in nature, distinct from that in the testis and only present on 4qA, but not on 4qB or 10q chromosomes [8, 9, 21]. 4qA and 4qB alleles differ by small but consistent polymorphisms in and around the D4Z4 repeat array and in the Caucasian population they are equally common [4]. Only when the *DUX4* transcript can make use of this somatic *DUX4* polyadenylation signal unique to 4qA, the D4Z4 chromatin relaxation leads to a higher probability of *DUX4* expression in skeletal muscle. This results in the molecular hallmark of the disease with sporadic nuclei expressing *DUX4* in FSHD myotube cultures [8, 9].

DUX4 is a double-homeobox transcription factor that binds to a double-homeobox motif and regulates the expression of genes associated with stem cell and germline development.

Mis-expression of DUX4 in skeletal muscle induces cell death and atrophic myotube formation [22–25] indicating that DUX4 expression is likely to be a major cause of the muscle pathology in FSHD. Furthermore, a large transcriptional deregulation that includes up-regulation of germline genes, genes required in stem cell biology as well as lncRNAs, and suppression of innate immune response genes has been reported in DUX4-transduced myoblasts as well as FSHD muscle [8, 26]. DUX4 binds to the long terminal repeat (LTR) sequence of a class of retrotransposons and approximately one-third of the genes regulated by DUX4 initiate transcription in these LTR sequences. In addition, DUX4 expression leads to the inhibition of nonsense-mediated RNA decay [27–29] and the accumulation of a large number of RNA transcripts that would normally be degraded. It is currently not clear whether altered expression of genes in a particular pathway is the major cause of FSHD pathophysiology or whether the disease state of the muscle is a consequence of disruption in many pathways. Some of the DUX4 target genes could turn out to be valuable biomarkers for disease diagnosis and progression. Interestingly, DUX4 expression as well as activation of some of the DUX4 target genes has been reported in fetal FSHD1 muscle tissue suggesting that some FSHD pathology might occur early in muscle development [30].

In cultured FSHD muscle cells, DUX4 is expressed in a small subset of nuclei, generally ~1% or fewer nuclei in the culture, likely representing occasional bursts of DUX4 expression [8]. Because myotubes are multinucleated, the DUX4 protein produced in the cytoplasm from the expression of *DUX4* mRNA from one nucleus can be imported to adjacent nuclei, spreading the activity of the DUX4 transcription factor to a larger region of the myotube [31]. The *DUX4* mRNA has the translation stop codon in the first exon and the polyadenylation signal in the third exon, making it a target of nonsense mediated decay [29], which might explain why the *DUX4* mRNA is present at such low abundance and has been difficult to detect. Interestingly, DUX4 expression results in decreased levels of the UPF1 protein, an essential component of the nonsense-mediated decay pathway. This creates a self-reinforcing loop since suppressing the nonsense-mediated decay pathway stabilizes the *DUX4* mRNA [29].

Loss of D4Z4 silencing in FSHD2 and discovery of SMCHD1

There is a less common form of FSHD, and these individuals diagnosed with FSHD2 display clinical features that are identical to those observed in FSHD1 patients [32]. However, a normal sized, albeit often somewhat shorter, D4Z4 repeat array on chromosome 4qA characterizes FSHD2. FSHD2 patients show a strong reduction of D4Z4 DNA methylation on both chromosomes 4 and 10 suggesting impaired epigenetic silencing of the repeat units, similar to the contracted D4Z4 repeat arrays in FSHD1 [5, 32]. FSHD2 patients carry at least one somatic *DUX4* polyadenylation signal-containing chromosome 4, and, like FSHD1 patients, display a variegated DUX4 expression pattern in muscle cell cultures [8].

A recent breakthrough in the field was the identification of mutations in the Structural Maintenance of Chromosomes Hinge Domain Containing 1 (*SMCHD1*) gene in FSHD2 patients using whole exome sequencing [5]. Several studies have since identified disease-causing variants in *SMCHD1* that include splice site, insertion-deletion, missense and nonsense variants [19, 33–38]. *SMCHD1* mutations are now presumed to account for ~85%

of FSHD2 cases [39]. In addition to its involvement in silencing of the D4Z4 repeat array, in mammals SMCHD1 has been implicated in several alternative silencing pathways. These include X-chromosome inactivation, silencing of monoallelically-expressed genes, and telomere silencing suggesting an important role for components of the repeat silencing machinery in FSHD [40–43].

SMCHD1 is a distant member of the highly conserved SMC protein family that form essential components of the cohesin/condensin protein complexes. In muscle cells SMCHD1 has been reported to directly bind to the D4Z4 repeat array, suggesting that it is required to maintain a repressive chromatin structure in somatic cells [5]. It has been shown that SMCHD1 binds to telomeres and a recent study reported increased SMCHD1 levels at long telomeres in human cell lines [44, 45]. A similar mechanism can be envisioned for SMCHD1 at the D4Z4 repeats where varying repeat unit size is associated with the amount of SMCHD1 binding. However, the precise molecular mechanism of SMCHD1 function at D4Z4 remains to be determined.

Intriguingly, SMCHD1 has recently also been identified as a modifier of disease severity in a growing number of FSHD1 families with upper-sized FSHD alleles of 7–10 D4Z4 units [33, 36]. In these situations, the combination of a contracted D4Z4 repeat array and a SMCHD1 mutation leads to an earlier disease onset and a more rapid disease progression. In muscle cell cultures, it correlates with increased DUX4 levels [33].

Balancing between genetic and epigenetic factors

One of the most intriguing clinical observations in FSHD is the marked inter- and intrafamilial variability in disease onset and progression [46]. While one-fifth of FSHD1 patients will become wheelchair dependent, an equal proportion of FSHD1 mutation carriers are asymptomatic but minimally affected on examination [47]. Moreover, approximately 1–2% of healthy control individuals carries a D4Z4 repeat array of 8–10 units on a 4qA allele, putting them at risk of developing FSHD [17, 18].

Early studies already suggested a rough and inverse relationship between D4Z4 repeat size and disease severity in FSHD1, with individuals carrying 1–3 units typically representing the most severe end of the disease spectrum [48–51]. Recent studies have, however, started to uncover some aspects of the molecular basis of this marked clinical variability in disease onset and progression. While initial epigenetic studies were only able to uncover differences in D4Z4 methylation between the shortest D4Z4 repeat arrays (1–3 units) and longer FSHD1 alleles [6], more recent studies have shown a correlation between D4Z4 repeat array size and D4Z4 methylation [19]. Moreover, several studies have uncovered differences in D4Z4 methylation between affected carriers and non-affected carriers of an FSHD1 allele [19, 52, 53]. This was most notable in disease alleles in the range of 8–10 units, where unaffected carriers had higher D4Z4 methylation levels than what might have been expected based on the repeat size [19]. This suggests that other, possibly polymorphisms or hyper- or hypomorphic variants in epigenetic modifiers, contribute to a D4Z4 chromatin state that protects against disease presentation in these unaffected carriers of an FSHD1 allele (Fig. 2).

This may also explain the high frequency of upper-sized FSHD1 alleles in the healthy control population.

Furthermore, SMCHD1 mutation studies have suggested that the effect of the SMCHD1 mutation on D4Z4 methylation and disease presentation depends on the D4Z4 repeat array size and on the consequence of the mutation on SMCHD1 protein levels or conformation (Fig. 2). Mutations that disrupt the open reading frame are believed to result in SMCHD1 haploinsufficiency and have less of an effect on D4Z4 methylation than mutations that preserve the SMCHD1 open reading frame and possibly act through a dominant negative mechanism [19]. Consequently, while SMCHD1 haploinsufficiency is typically found in FSHD2 patients with a D4Z4 repeat array of 11–16 units, mutations that exert a dominant negative effect can also cause FSHD with longer D4Z4 repeat arrays. Thus, alleles of 8–16 D4Z4 units are more susceptible to chromatin relaxation as a consequence of SMCHD1 mutations than longer arrays. This may explain the observation that the FSHD2 population carries on average smaller D4Z4 repeat arrays than the normal control population and suggests that the susceptibility to somatic DUX4 expression and disease presentation is dependent on a combination of genetic and epigenetic risk factors.

Conclusions

In recent years major advances in our understanding of the molecular mechanisms underlying FSHD have been made. This has led to a model positioning DUX4 as a central player in FSHD pathophysiology. Also, the identification of *SMCHD1*, as the disease gene for FSHD2 and its role as a disease modifier in FSHD1, has highlighted that imbalances between genetic context and epigenetic regulation can crucially impact disease development. Nevertheless, there are a number of questions that remain unanswered. Given that the clinical features of FSHD usually appear during the second decade of life and are marked by a high intrafamilial clinical variability it will be important to determine how this ‘disease state’ of the muscle is reached. It will also be vital to further define the genetic and epigenetic signatures that could be predictive of disease onset, progression or severity. A better understanding of the components and mechanisms of D4Z4 silencing will be crucial to advance epigenetic-based therapeutic development in FSHD.

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Figure 1. Genetic basis of FSHD1 and FSHD2

Two genetic forms of FSHD are known. The most common form, FSHD1, is caused by contraction of the D4Z4 repeat array to a size of 1–10 units. In controls the D4Z4 repeat arrays (triangles) vary between 11–100 units and adopt a repressed chromatin structure (brown) in somatic cells such as skeletal muscle. CEN indicates the centromeric end and TEL indicates the telomeric end. Upon contraction the D4Z4 chromatin structure becomes derepressed (green) facilitating the expression of the *DUX4* retrogene of which a copy is embedded within each unit. *DUX4* (white boxes with open reading frame in black) within the D4Z4 unit does not have a polyadenylation signal (PAS), but, in somatic cells can make use of a polymorphic PAS immediately distal to the D4Z4 repeat present on 4qA but not on 4qB chromosomes. Thus, only on 4qA chromosomes, D4Z4 chromatin derepression leads to the production of DUX4 protein.

SMCHD1 is a chromatin modifier that binds to the D4Z4 repeat to keep the D4Z4 chromatin structure in a repressed state in somatic cells. Individuals with a mutation in SMCHD1 (asterisk), in combination with a *DUX4* polyadenylation signal, can express DUX4 in their muscles. This is called FSHD2.

Figure 2. D4Z4 repeat array sizes in FSHD1 and FSHD2

Recent studies indicate a gradient of probability of disease presentation with increasing repeat sizes. While individuals with 1–3 units on a FSHD-permissive (*DUX4* polyadenylation signal-containing) allele are almost exclusively severely affected early onset cases, individuals with 4–10 units show much more clinical variability ranging from severely affected individuals to asymptomatic gene carriers. In part, this variability can be explained by differences in epigenetic susceptibility to somatic expression of *DUX4*. This is best exemplified in *SMCHD1* mutation carriers. *SMCHD1* can act as disease gene in FSHD2 or as modifier gene in FSHD1, depending on the consequence of the mutation. Mutations that lead to *SMCHD1* haploinsufficiency only cause FSHD in combination with intermediate sized D4Z4 repeat arrays of 8–16 units, while *SMCHD1* mutations that are likely to exert a dominant negative effect, can cause FSHD also with longer D4Z4 repeat arrays.