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REVIEW

Deciphering Facioscapulohumeral Dystrophy in the clinical trials era: where are we now?

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Objectives. Facioscapulohumeral muscular dystrophy (FSHD) is a common genetic disorder characterized by progressive muscle weakness, especially in the face, shoulders, and upper limbs. Despite extensive research, the underlying pathogenesis and clinical variability remain incompletely understood. This review aims to summarize recent advances in FSHD research, focusing on genetic and epigenetic factors and the potential for precision medicine.

Methods. A comprehensive review of recent literature was conducted, examining molecular mechanisms such as mutations in the D4Z4 region, DUX4 expression, RNA interference (RNAi) and antisense oligonucleotides (AOs). Clinical variability was analyzed to assess different disease phenotypes. Clinical trials investigating potential treatments, especially those targeting DUX4, were also reviewed.

Results. FSHD shows significant clinical variability, with different progression rates across phenotypes. The 4qA allele is linked to more typical forms of the disease, but epigenetic factors, including DNA methylation and miRNA expression, also influence disease severity. Despite progress, the exact molecular mechanisms driving disease expression remain unclear. Clinical trials, such as Losmapimod, show promise in slowing muscle degeneration, though results remain inconsistent.

Conclusions. FSHD presents significant challenges for therapy development due to its genetic complexity and clinical variability. Ongoing research is needed to clarify pathogenesis and identify reliable biomarkers. Future therapeutic strategies should focus on precision medicine, integrating genetic, clinical, and imaging data to optimize patient stratification and treatment efficacy.

Keywords: Facioscapulohumeral muscular dystrophy (FSHD), DUX4, Clinical variability, Therapeutic strategies, Clinical trials

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Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common forms of inherited muscular dystrophy overall, with an estimated prevalence of 1:8.333[1]. FSHD is characterized by a selective pattern of skeletal muscle weakness, however a wide spectrum of disease expression is commonly observed. The clinical variability mirrors the genetic complexity of disease. While evidence is growing about phenotypical variability and possible different disease courses, knowledge of pathophysiologic mechanisms underlying those differences is still far from comprehensive. As clinical trials are finally approaching also for FSHD, understanding of the interplaying factors determining disease course, phenotypic characterization of patients, and choosing appropriate outcome measures is

crucial for correct selection of molecules, trials' design and selection of endpoints.

Molecular basis of disease

The pathogenic molecular mechanism is believed to reside in an aberrant expression of the Double homeobox 4 (DUX4) gene due to hypomethylation in the D4Z4 region on chromosome 4q35. *DUX4* (Fig. 1) is a double-homeobox transcription factor that is normally expressed during embryogenesis and activates a core set of genes involved in zygotic development; it is then silenced in most adult tissues except thymus and testis [2,3]. Two major 4q allelic variants exist (4qA and 4qB), but only the 4qA allele, therefore named "permissive", presents a polymorphic DUX4 polyadenylation signal enabling DUX4 expression [4,5], which leads to a cascade of pathological processes including inflammation [6], altered cells differentiation [7,8-10], impaired transcription quality [11,12,13] and DNA repair control [7,14], mitochondrial dysfunction with increased susceptibility to oxidative stress and finally cell death [7,15,16]

About 95% of subjects with clinical diagnosis of FSHD (FSHD1) are carriers of a reduced length of the D4Z4 region which is generally represented by a number of 11-100 tandemly arrayed repetitive DNA elements (DRAs) in most of normal population. In particular, FSHD1 is associated to a reduced number of DRAs ranging from 1-10 repeat. This repeat has a high CG dinucleotides content making it highly susceptible to transcriptional repression through DNA methylation [17,18,19].

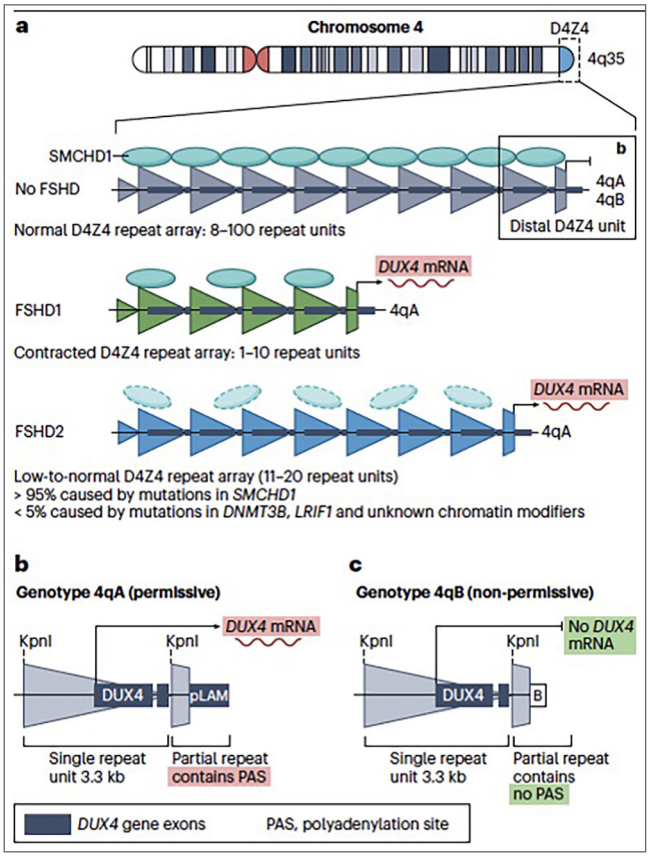

Figure 1. The D4Z4 repeat array on chromosome 4q in healthy individuals and patients with facioscapulohumeral muscular dystrophy [30].

The reduced number of repeats leads to loss of D4Z4 DNA methylation, which turns into chromatin relaxation, and successively in the aberrant expression of DUX4 gene in skeletal muscle [20,21]. Notably, beyond the DRAs and the open chromatin structure, the presence of at least one permissive haplotype of the 4q subtelomeric region is necessary to allow DUX4 expression [22]. The remaining minority of patients, termed FSHD2, do not carry DRAs number reduction on D4Z4, although presenting similar if not greater alterations of chromatin relaxation at the same 4q35 region [22], with a main difference, however, being chromatin relaxation on both D4Z4 copies, whereas hypomethylation in FSHD1 is only observed in the contracted allele. In FSHD2 patients, pathogenic mutations in other genes are reported. Most of the variants associated to FSHD are found in Structural Maintenance of Chromosomes flexible Hinge Domain-containing protein 1 (*SMCHD1*) gene, that encodes an epigenetic regulator that promotes and maintains the heterochromatin compaction status at the D4Z4 locus [23,24,25]. Other genes associated to FSHD2 are: DNA Methyltransferase 3 Beta (*DNMT3B*), that is a DNA methyltransferase, and Ligand Dependent Nuclear Receptor Interacting Factor 1 (*LRIF1*) gene, which codes for an interactor of SMCHD1, has been reported [26,27]. These genes play a key role in maintaining epigenetic repression at the D4Z4 locus when a permissive 4qA subtelomere is present. Notably, *SMCHD1* mutations can also be present in association to reduced DRAs in FSHD1 and in this case it seems to act as disease modifier [28,29]. In summary, in both FSHD1 and FSHD2 chromatin relaxation leads to the aberrant de-repression of DUX4 gene expression. This evidence suggests that FSHD1 and FSHD2 should not be considered as distinct pathologies but parts of the same disease continuum.

In recent years, several observations are challenging the traditional knowledge on molecular mechanisms involved in disease expression and severity in FSHD, as they do not completely explain the incomplete penetrance of disease among families and the presence of different phenotypes and natural history courses [30]. Incomplete silencing of the FSHD locus results in the misexpression of the pathogenic DUX4 transcript in skeletal muscles. This leads to the activation of an embryonic gene expression program by DUX4, which in turn initiates numerous downstream aberrant events. Although some of the pathways downstream of DUX4 can be targeted, it remains unclear which specific pathway, if any, is directly responsible for the disease pathology [31]. As a result, significant efforts to develop targeted FSHD therapies have concentrated on re-establishing silencing at the locus or inhibiting the expression of DUX4 mRNA or protein.

Indirect expression pharmacological screening using DUX4 reporter constructs has enabled the identification of several compounds that repress the expression of DUX4, such as bromodomain and extra-terminal (BET) inhibitors, β2 adrenergic receptor agonists, phosphodiesterase (PDE) inhibitors, p38 inhibitors and Wnt pathway agonists [32-36]. In a different approach, a genome-wide CRISPR-Cas9 screen identified DUX4 pathways that could potentially be targeted for therapy. In recent years, multiple research teams have explored CRISPR-based modulation of the FSHD locus, utilizing either Cas9-mediated gene editing (CRISPRe) or dCas9-mediated transcriptional repression (CRISPRi). However, studies conducted by various groups on editing the DUX4 polyadenylation signal (called PLAM), that is located outside of the D4Z4 region in the 4qA variant

of chromosome 4 and is necessary for the production of the DUX4 protein, have yielded conflicting conclusions regarding the reduction of pathogenic DUX4 levels [37].

Campbell et al showed that the regulation of DUX4 involves BET proteins, particularly (Bromodomain-containing protein 4) BRD4, and the beta-2 adrenergic signaling pathway. BRD4 binds to acetylated lysines in the D4Z4 array and recruits complexes such as P-TEFb and Mediator to facilitate transcription by RNA polymerase II. BET inhibitors (BETi) block BRD4 binding with consequently reducing DUX4 expression. Additionally, activation of the beta-2 adrenergic receptor by an agonist stimulates adenylyl cyclase, increasing cAMP levels. Although cAMP activates multiple pathways, the inhibitory effect of beta-2 agonists on DUX4 expression appears to occur mainly through PKA-independent mechanisms. These may involve signaling molecules such as phosphatases (PPtases) and MAPKs, which influence chromatin modifiers like lysine methyltransferases (KMTases) to regulate DUX4 transcription [32]. Like the β2 adrenergic receptor, the PDE inhibitor has been explored as a potential modulator of DUX4 activity. Inhibition of PDEs contributes to the stabilization and accumulation of cAMP. Cruz and colleagues investigated ibudilast and crisaborole, two already approved PDE inhibitor drugs, to assess their ability to inhibit DUX4-dependent gene expression. Both inhibitors led to a significant decrease in *DUX4* mRNA levels, with reductions of 83% and 76%, respectively, compared to FSHD myotubes treated with DMSO. The influence of these two pathways on lowering DUX4 and DUX4-dependent gene expression indicates the involvement of cAMP-mediated signaling in this process [33]. An example of kinases activated by β2 adrenergic signaling is the group of p38 mitogen-activated protein kinases (MAPKs). The p38 MAPKs are classically involved in the cellular response to stressful stimuli, including inflammatory cytokines, and have been extensively pursued by pharmaceutical companies for diseases with inflammatory components. The study conducted by Rojas and colleagues and Oliva and colleagues demonstrated that *DUX4* mRNA synthesis in FSHD myoblasts and myotubes is extremely sensitive to p38α/β inhibitors. However, the molecular mechanisms that link *DUX4* expression to p38 activity remain to be elucidated [34,35].

The Wnt/β-catenin signaling pathway has been implicated in FSHD pathology due to its role in muscle development and the organization of facial muscles [38]. Wnt genes encode a family of secreted proteins that play a role in many aspects of embryonic development and tissue homeostasis through the activation of receptor-mediated signaling pathways [39,40]. Block et al focused on the effect of Wnt/β-catenin signaling on *DUX4* expression and for the first time showed that activation of the Wnt/β-catenin pathway in FSHD myotubes leads to a reduction in *DUX4* expression levels and prevents DUX4-dependent myotube apoptosis. Block and colleagues demonstrated that the reduction of transcripts encoding Wnt pathway components results in *DUX4* activation, consistent with the hypothesis where *DUX4* transcription is subject to active Wnt-mediated suppression [36].

Moreover, suggestions for the possible role of other genetic and epigenetic factors come from growing evidence in recent literature. Among these Grow Ej and colleagues found that in FSHD1 and FSHD2 cells, DUX4 is hypersensitive to p53 activation, unlike in non-FSHD cells. This is likely due to the relief of chromatin/epigenetic silencing, which occurs specifically in FSHD genotypes. This mechanism may explain the stochastic expression of DUX4 in muscle cells from FSHD patients, who are known to exhibit DNA damage [41]. Moreover, apoptosis in skeletal muscle cells, that is the most severe consequence of DUX4 expression. P53 is a component of the final common pathway of DUX4-mediated apoptosis, although the molecular mechanism remains unclear [42].

Moreover, it is worth mentioning the matter of selecting the most suitable cellular model and how to collect human cells to test pathogenetic hypotheses: in fact, development of therapeutic options for the disease is further complicated by the difficulties of replicating reliable FSHD human pathology in vitro and in animal models. A first difficulty comes from the presence of D4Z4 array and DUX4 gene only in Old World Primates [45], while the murine Dux gene is not completely superposable in sequence and function to DUX4 [46]. Currently, the two recently developed and used mouse models for FSHD consist of exogenic expression of human DUX4 in transgenic animals by means of vary systems in one case (AAV-6 DUX4, TIC DUX4, iDUX4; and in human-to-mouse muscle tissue or cells xenograft. The first method provides the possibility to regulate DUX4 expression, although not respecting the muscle selective pattern that characterizes human disease and not representing a specular model for D4Z4 region structure and epigenetic interactors [47] ;the second one ensures conservation of the human D4Z4 array integrity and reliably reproduces human pathology in vivo, serving as the best method for therapeutic testing, while not permitting functional evaluations and altering the immune system landscape, so that study of this possible disease contributor is not possible [48].

Taken together, these findings underscore the paramount importance to phenotypically characterize patients with FSHD and used standardized methodologies, in order to analyze the various possible disease modifying factors in homogenous populations on one side; on the other, they highlight the complexity of FSHD, that requires the study of multiple biological and mechanistic perspectives in order to deepen our understanding.

Phenotypic Variability

The disease, firstly described in 1885 by Landouzy and Dejerine, is typically characterized by young or adult-onset progressive facial, shoulder girdle and pectoral muscle weakness and atrophy, subsequent involvement of abdominal muscles - manifesting with lumbar hyperlordosis, prominent abdomen and positive Beevor's sign, a physical finding fairly specific for FSHD - and of anterior leg muscles with steppage gait and foot drop. Pattern of weakness distribution is typically asymmetric and progression is slow over time, with males presenting a lower mean age at clinical onset compared to females. Patients with shorter allele can manifest an early-onset in childhood with more severe progressive motor impairment, the most likely also to display extra muscular involvement like exudative retinopathy known as Coat's disease, sensorineural deafness and cognitive delay [49-52]. Cardiac involvement is not typical as well as ventilatory issues that, while not directly dependent from respiratory muscles involvement, can derive from spine and abdominal weakness. Nevertheless, FSHD patients can display a wide clinical variability, even

within the same family in which segregates the same size of allele, as describe initially by Padberg [53], ranging from asymptomatic to severe phenotype. In the recent years, the growing rate of assessment of the D4Z4 array size has increased the diagnostic yield and led to the identification of phenotypes that differ at various degrees from the aforementioned description. For example, there are several reports of cases that carry DRAs but show no facial weakness [54-56], defined facial sparing phenotype. Another frequently described atypical phenotype included bent spine syndrome, isolated or in association with other more classical features [57]. Interestingly, most of those patients were sporadic cases. Also, cases of cardiac involvement, including hypertrophic cardiomyopathy, conduction defects and arrhythmias, have been reported in subjects carrying a reduced allele [58-61]. The Italian Network for FSHD described large and well-characterized cohorts of FSHD patients by using the Comprehensive Clinical Evaluation Form (CCEF) [62]. The CCEF has been created to define various clinical categories of FSHD phenotypes through the combination of different features, by classifying subjects with typical facial and scapular girdle muscle weakness (category A, subcategories A1-A3), subjects in whom muscular involvement is limited to scapular girdle or facial muscles (category B subcategories B1, B2), asymptomatic subjects (category C, subcategories C1, C2), and subjects with a non-FSHD myopathic phenotype (i.e. isolated bent spine syndrome) (D, subcategories D1, D2). The CCEF was firstly applied by the Italian Clinical network in the following studies. Ruggiero and coworkers [63] found large phenotypic variability associated with individuals carrying a DRA with 7 to 8 repeated units, reporting that 47.1% of probands did not display the classic FSHD phenotype. Ricci et al (2020) [64] highlighted the high clinical variability among carriers of borderline 7-10 DRAs (bDRAs) with a frequent involvement of axial and pelvic muscles in patients with atypical phenotypes, features that can be found also in other inherited and acquired myopathies such as inflammatory, congenital or metabolic myopathies and limb-girdle muscular dystrophies [64]. Vercelli et al in 2021 [65] reported a 5-years longitudinal clinical follow-up in 246 DRA carriers. The study demonstrated a high predictive value of the CCEF categories for progression of disability - faster for category A subjects in comparison to patients with a facial-sparing phenotype (category B1).

Similar observation derived also from the UK FSHD Registry study that applied the CCEF to retrospectively characterize patients recruited in the registry [66,64], providing the hypothesis of a milder phenotype in patients without facial involvement (clinical category B2).

The development of facial weakness appears as a typical feature of FSHD and has been supposed to run on its own binary: Loonen et al (2020) described a cohort of more than eighty patients, in which a very mild facial weakness was observed in patients with bDRAs and more severe involvement in carriers of short alleles, but no correlation was observed between the degree of facial weakness and the duration of the disease. Their findings suggest that facial weakness may represent a distinctive feature in some patients, that manifest this sign since the beginning of disease without later significant progression. On the same basis, the facial-sparing phenotype should not represent an "initial" phase of disease but a phenotypical subgroup characterized by a different disease progression rate [67].

Overall, the significance of the different clinical forms for prognostic prospective and diagnosis/genetic counseling is still not always clear in clinical practice, although in literature growing evidence suggests the need to consider this variability. The different phenotypes could show a different disease progression and/or imply distinct genetic mechanisms. Moreover, the high heterogeneity of symptoms in association with a variable penetrance further complicate the genetic diagnosis, as well as the genotype-phenotype correlations, considering the complex genetic architecture to be considered. An agreement on a sharing and standardized clinical evaluation should be reached in the community of clinicians and researchers involved in FSHD.

Clinical trials, outcome measures and biomarkers

To recapitulate the challenges in therapy development in FSHD, we must consider the clinical variability and reduced penetrance of disease among carriers of a similar genetic signature, the lack of standardization of recruited populations in clinical trials, the absence of a comprehensive preclinical model to test molecular and therapeutic hypotheses on and the difficulty of identifying easily accessible and reliable biomarkers reflecting disease variability and progression. To date, there are no approved treatments for FSHD and standard of care only implies personalized physical therapy and management of motor disability. The accepted hypothesis involving aberrant DUX4 expression in skeletal muscle, has guided the research pipeline for targeted therapies, in particular treatments reducing DUX4 gene expression. Several clinical trials have employed non-FSHD specific (or targeting downstream effects of pathology as mitochondrial dysfunction) molecules. For instance, antioxidants have been tested based on the increased oxidative damage that is evident upon DUX4 expression, but only led to slight increase in strength and endurance of quadriceps [68]; two trials on myostatin have been conducted and then discontinued for lack of improvement compared to placebo [69,70]; albuterol, a beta2-adrenergic receptor agonist, failed to show any improvement in muscle strength in a double-blind placebo-controlled clinical trial [71]. Losmapimod, a p38-mitogen activated protein kinase inhibitor that has been studied in multiple fields from oncology to cardiovascular medicine, has been proposed as a novel treatment for FSHD and a phase III clinical trial is currently recruiting patients. Losmapimod has shown in preclinical studies robust capacity of inhibiting DUX4 and downstream genes' activation [72-35] and the phase I trial demonstrated general safety and effective p38 inhibition on muscle biopsies from several sites (vastus lateralis and medialis, gastrocnemius lateralis and medialis, tibialis anterior) from both STIR+ and negative muscles at MRI, and blood. These results led to a phase 2 clinical trial (ReDUX4) with subsequent open label extension. In ReDUX4, the primary endpoint (change of DUX4-induced gene expression) in muscle biopsies was not met, although at the end of the 48-weeks trial a slowing in muscle tissue fat infiltration at muscle MRI was reported along with significant improvement in the Reachable Work Space clinical outcome measure. Despite not reaching the primary endpoint, based on these data, the phase III trial has been approved. When evaluating these results, it is important to also consider the non-disease specific effects of Losmapimod, like the anti-inflammatory action, as the p38 MAPK cascade plays a pivotal role in initiation and progression of

inflammatory pathways and activation of pro-inflammatory cytokines, and vasoregulatory effect in improving microcirculation [73].

As to design and interpretation of clinical trials for FSHD, lack of homogeneous stratification of patients may raise the risk to test the same molecule acting on a precise pathogenic mechanism that may not be equally involved in all patients; moreover, selection of outcome measures acquires paramount importance, may they be clinical scales or biomarkers (biochemical as methylation levels and others or radiological as muscle MRI), and this process should be preceded by proper clinical characterization patients. In general, outcome assessments in FSHD natural history studies or clinical trials include clinical parameters collected by clinicians, patient-reported outcomes with clinicians- or self-assessed questionnaires, and biomarkers. The ReDUX4 trial is representative in this sense, as considered outcome measures were gene expression in tissues, whole body quantitative muscle MRI, digitally (Reachable Work Space, hand-held dynamometry) and manually (Motor Function Measure, Timed Up and Go) assessed clinical scales and motor tasks and quality of life questionnaires (Patient's Global Impression of Change, FSHD Health Index). Reachable Work Space (RSW) in particular is based on the use of a motion sensor (Kinect) providing a representation of the patient's upper limbs reachable area; it proved to be sensitive in detecting changes from baseline and, in a 5-years long longitudinal studies, demonstrated two clinical subgroups characterized by a different disease course [74]. Notably, in this study the subjects were not subdivided based on disease phenotype and included severity degrees from 1 to 15 points on the FSHD Evaluation Scale, reflecting a range encompassing paucisymptomatic forms to severe disability. Recently, a study by Tasca (2022) retrospectively identified a machine-learning developed muscle MRI involvement pattern that displays a high specificity for FSHD, including trapezius abnormalities and subscapularis and ileopsoas sparing and asymmetric involvement as main features. Patients recruited included an 18% of atypical cases, defined so in case of one uncommon clinical feature as camptocormia, dropped head or predominant pelvic girdle weakness, and the identified MRI alterations permitted identification also in more than 90% of those cases. While providing a possibly important diagnostic biomarker, it would be interesting to evaluate the presence of longitudinal changes in clinically different subgroups [75]. As to circulating biomarker, Sacconi et al demonstrated a correlation between levels of concentration of IL-6, an inflammatory marker, and clinical parameters of disease severity (muscle manual testing, Vignos score, Brooke score, Clinical Severity Scale) [29]. Again, patients were not stratified based on phenotypic characteristics but only on disease severity, while it would widen our comprehension to analyze this biomarker in diverse phenotypes with longitudinal follow-up.

Other clinical trials are underway to develop effective treatments for facioscapulohumeral muscular dystrophy (FSHD). miRNAs have also been proposed as disease biomarkers; several studies identify altered expression of variable numbers of miRNAs targeting factors involved in myogenesis and muscle function compared to control cells, although with different results [76-79]. FSHD is a dominant gain-of-function disease, particularly well-suited for antisense or RNAi-based approaches. Over the past decade, various antisense strategies have been tested against the DUX4 gene and its pathogenic transcript, achieving significant success in both in vitro and in vivo proof-of-principle studies. The initial antisense research in this field concentrated on modifying DUX4 pre-mRNA processing. In healthy cells, DUX4 can generate a shorter mRNA isoform, which is translated into a non-toxic protein. However, in FSHD myocytes, a shift in mRNA splicing occurs, leading to the production of the full-length, pathogenic DUX4 isoform [10,80] Notably, a study demonstrated that systemically administered Phosphorodiamidate morpholino oligomers (PMOs) targeting the polyadenylation signal (PAS) region effectively reduced DUX4 levels and its downstream targets, alleviated pathological features, and enhanced muscle function in the FLExDUX4 FSHD mouse model [4,81].

It is encouraging that a number of biotech companies (Avidity Biosciences, Dyne Therapeutics, Arrowhead Pharmaceuticals, miRecule, and Armatusbio) are actively developing DUX4-targeting oligonucleotide therapeutics for FSHD. The targeted oligonucleotide therapies are divided into: RNA interference (RNAi) and antisense oligonucleotides (AOs) [82].

RNAi is a conserved biological mechanism in which double-stranded RNA triggers the degradation of homologous mRNA. Through this pathway, RNAi-based oligonucleotides operate at the RNA level by binding to their target mRNA via antisense sequence complementarity, thereby initiating post-transcriptional gene silencing. The process begins when DICER endonucleases cleave precursor molecules, such as pre-miRNA or short hairpin RNA (shRNA), generating mature miRNA or siRNA. These RNA fragments are then incorporated into the Argonaute (AGO) protein within the RNA-induced silencing complex (RISC). RISC binds to the target mRNA and either inhibits its translation [83,84].

Two RNAi-based oligonucleotide therapies for FSHD are currently recruiting for Phase I/II clinical trials to evaluate their safety, tolerability, pharmacokinetics, pharmacodynamics, and efficacy in adult patients. Both therapies have previously demonstrated preclinical efficacy in cellular and murine models of FSHD.

Other possible disease modifying factors at study are long non coding RNAs (lncRNAs), which are involved in multiple biological mechanisms including regulation of transcription, enzymatic activity, cell differentiation and which have been found to be altered in a number of human disorders; in particular the lncRNA DBE-T, that has a transcription initiation site proximal to the D4Z4 repeat, was shown to be upregulated in FSHD and involved in derepression of DUX4 through the recruitment to the D4Z4 region of the Trithorax group protein ASH1 [85]. Currently, the EJPRD-Epi4FSHD project is underway, in which WDR5 has been identified. WDR5 binding to specific lncRNAs is essential to maintain active chromatin. The main hypothesis is that blocking WDR5 protects the cell from DUX4-induced toxicity by reducing the transcriptional activation of DUX4 and its target genes. This approach will be tested on a preclinical FSHD model, specifically focusing on parameters such as cell growth, apoptosis, and myoblast differentiation [86]. To do that, primary muscle cells are being collected from affected FSHD patients and healthy relatives through needle muscle biopsies. These cells will be treated with different WDR5i concentrations and expression of DUX4 and its target genes will be monitored by RT-qPCR to determine the effective WDR5i concentration. Different amounts of exposure times will also be tested.

Other studies have explored the possible role of histones modifications as disease biomarkers and in correlation with disease severity;

in particular, a study by Balog et al examined the ratio between the levels of histones' methylation H3K9me3, marker of transcriptional repression, and of H3K4me2, marker of open chromatin, considering this ratio to be related to the level of chromatin compaction, which they found to be decreased in cells from patients with respect to controls, although failing to confirm the correlation with clinical severity on myoblasts [87]. Finally, the possible role of DUX4 associated genes like PAX7 as biomarkers of disease in the absence of a spatial and temporal reduced availability of DUX4 expression evidence in patients' tissues and arguable correlation with clinical features, has been explored. Banerji et al (2020) demonstrated the correlation between PAX7 expression and clinical severity progression over a 1-year long follow-up in a superior manner compared to DUX4 (as previously claimed by Wang et al), in cells obtained from MRI-informed biopsies from FSHD patients [88]. Notably, PAX7 is also known as a regulator of fibro-adipogenic progenitor cells, which have been recently proposed as further players in the landscape of FSHD pathomechanisms [89]. Nonetheless, consensus is far from reached: a recent study by van den Heuvel (2022) on muscle biopsies obtained from TIRM+ and TIRM-lower limb muscles (Tibialis Anterior and Vastus Lateralis) of FSHD patients shows variability of the genetic signatures even in biopsies obtained from two contiguous sites of the same muscle showing abnormalities on MRI, no clear correlation between the DUX4 signature and PAX7 expression, and the risk of missing DUX4 or PAX7 expression by selecting only STIR/TIRM+ muscles for biopsy, as positivity of the two signatures was present also in muscles with no abnormalities at MRI. Overall, based on their data, the authors suggest that DUX4 may be considered as a biomarker in TIRM+ muscles and PAX7 in muscles with more advanced pathology. In the cited studies, there was no uniformity in patients' clinical severity and phenotype nor in selection of muscles, which may contribute to the variable obtained results [89].

Can we do better?

Although being one of the most common muscular dystrophies, known for decades by clinicians, complete and clear knowledge on FSHD pathogenic mechanisms and determinants of clinical variability among patients and families is still lacking. Clinical trials era has come, also for neuromuscular diseases, and profound understanding is needed in order to work on the right molecular targets, correctly design clinical trials and provide patients with the most suitable treatment for their disease. In the case of FSHD, this could mean we should also focus on clinical features for guidance towards a phenotype-based approach to precision and personalized medicine, from diagnosis to treatment. In fact, a conceptual model encompassing molecular characteristics (genetic and epigenetic) and phenotype is needed to provide the right basis to build clinical, translational and pharmaceutical research on.

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Authors contributions

FT BC MR conceptualization and writing, AV EM FK writing, ML EF MF DG GS GR FT supervision and approval.

Ethical consideration

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