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## A Pediatric Review of Facioscapulohumeral Muscular Dystrophy

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### Abstract

Facioscapulohumeral dystrophy is one of the most common forms of muscular dystrophies worldwide. It is a complex and heterogeneous disease secondary to insufficient epigenetic repression of D4Z4 repeats and aberrant expression of *DUX4* in skeletal muscles. Type 1 facioscapulohumeral muscular dystrophy (FSHD) is caused by contraction of D4Z4 repeats on 4q35, whereas type 2 FSHD is associated with mutations of the *SMCHD1* or *DNMT3B* gene in the presence of a disease-permissive 4qA haplotype. Classical FSHD is a slowly progressive disorder with gradual-onset of muscle atrophy and a descending pattern of muscle weakness. In contrast, early-onset FSHD is associated with a large deletion of D4Z4 repeats and a more severe disease phenotype, including early loss of independent ambulation as well as extramuscular manifestations, such as retinal vasculopathy, hearing loss, and central nervous system (CNS) involvement. However, the correlation between D4Z4 repeats and disease severity remains imprecise. The current standard of care guidelines offers comprehensive assessment and symptomatic management of secondary complications. Several clinical trials are currently underway for FSHD. New and emerging treatments focus on correcting the transcriptional misregulation of D4Z4 and reversing the cytotoxic effects of *DUX4*. Other potential therapeutic targets include reduction of inflammation, improving muscle mass, and activating compensatory molecular pathways. The utility of disease-modifying treatments will depend on selection of sensitive clinical endpoints as well as validation of muscle magnetic resonance imaging (MRI) and other biomarkers to detect meaningful changes in disease progression. Correction of the epigenetic defects using new gene editing as well as other *DUX4* silencing technologies offers potential treatment options for many individuals with FSHD.

### Keywords

facioscapulohumeral muscular dystrophy; review; new emerging treatments

## Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is a progressive genetic myopathy with variable onset of symptoms, distribution of weakness, and disease severity, as recently reviewed by several authors, including Tawil et al,<sup>1</sup> Sacconi et al,<sup>2</sup> Daxinger et al,<sup>3</sup> and Knopp et al.<sup>4</sup> It is one of the most common types of muscular dystrophies with an estimated incidence of 1 in 15,000<sup>5</sup> and a prevalence of 1 in 8,000 to 20,000 in the general population.<sup>6,7</sup> Up to 30% of genetically confirmed cases of FSHD can be asymptomatic,<sup>8</sup> especially among those with smaller deletions (8 to 10 copies of D4Z4 repeats).<sup>9,10</sup> Mosaic mutations may also be associated with a milder phenotype.<sup>11</sup> In contrast, infantile FSHD is an uncommon but more severe spectrum of FSHD. The purpose of this article is to provide a pediatric review of FSHD, including the diagnosis, standard of care, and new emerging treatment for children and adults with this disease.

## Clinical Features

Symptoms of classical FSHD typically present during the second or third decade of life with facial weakness, scapular winging, as well as limitation in shoulder abduction and elbow flexion (see ►Fig. 1). Asymmetric muscle involvement is common.<sup>12</sup> Progression of weakness is usually slow and follows a descending pattern with gradual involvement of the trunk, hip girdle, and distal lower extremities muscles. Over time, most affected individuals have a constellation of clinical signs related to FSHD, including eye closure weakness, reduced facial expression, transverse smile, inability to whistle or suck from a straw, periscapular muscle atrophy, high riding scapula, horizontal clavicles, Beevor's sign, increased lumbar lordosis as well as hip girdle and foot extensor weakness.<sup>13–15</sup> Atypical features, with relative facial sparing, calf hypertrophy, and predominantly lower extremities weakness, have been described.<sup>16–18</sup> In general, dysphagia,<sup>19</sup> cardiac arrhythmias,<sup>20</sup> cardiomyopathy,<sup>21</sup> and severe respiratory insufficiency<sup>22</sup> are uncommon complications in classical FSHD; additional investigations may be required to exclude non-FSHD-related or double-trouble etiologies.<sup>17,23</sup> Individuals with FSHD have an increased risk of developing chronic musculoskeletal pain and fatigue.<sup>16,24</sup> Life expectancy is unaffected; however, between 10 to 30% of individuals may become nonambulatory due to progressive muscle weakness.<sup>25</sup>

## Early-Onset FSHD

Early-onset FSHD makes up approximately 4 to 21% of the total FSHD population<sup>26,27</sup> and nearly half of the pediatric FSHD population;<sup>27</sup> the rest have the classical phenotype of late adolescent or adult onset disease. The term “infantile FSHD” was initially coined by Brooke in 1977 to denote an early-onset form of the disease.<sup>28</sup> The diagnostic criteria for infantile FSHD was further defined by Brouwer et al<sup>29</sup> to include affected individuals with onset (signs and symptoms) of facial weakness before 5 years of age plus sign and symptoms of shoulder girdle weakness before 10 years of age. In addition to facial diplegia and shoulder weakness, affected children may present with generalized hypotonia, global developmental delay, dysarthria, and dysphagia shortly after birth.<sup>30</sup> Rarely, features resembling Mobius syndrome with ophthalmoplegia have been described.<sup>31,32</sup> Additional manifestations, such

as retinal vasculopathy,<sup>33</sup> intellectual disability, epilepsy,<sup>34</sup> and cochlear dysfunction,<sup>35</sup> are more common among individuals with early-onset FSHD and large D4Z4 deletions. On occasion, these extramuscular features may be the initial presenting complaints, and thus, are particularly noteworthy for pediatric neurologists as potentially early signs of FSHD.<sup>27,29,30,36</sup> Prompt recognition is important for optimal treatment and family counseling.

Early-onset FSHD is generally associated with fewer number of D4Z4 repeats, younger age at loss of independent ambulation, and a greater risk of disease severity.<sup>27,30,37,38</sup> However, the correlation between disease severity and D4Z4 repeat number is inconsistent; infantile FSHD may be influenced by other genetic and environmental modifiers.<sup>39,40</sup>

## Pathogenesis

FSHD results from a highly unusual and complex disease mechanism, in which the mutation does not reside within the gene or genes responsible for the disease. Type 1 FSHD (FSHD1, Online Mendelian Inheritance in Man (OMIM) #158900; <https://www.omim.org/entry/158900>) is an auto-somal dominant disorder associated with deletions of integral copies of a 3.3 kb tandem repeat unit termed D4Z4 in the subtelomeric region of chromosome 4q35.<sup>41</sup> In FSHD1 patients, there are only 1 to 10 copies of D4Z4 repeats at the 4q35 region, while 11 to 100 copies are found in the normal population. Studies showed that the deletion causes DNA hypomethylation of D4Z4 repeats and transcriptional derepression of the region.<sup>42</sup> Less than 5% of individuals with FSHD have normal-sized repeat array on chromosome 4 but still exhibit DNA hypomethylation of D4Z4 repeats and carry a permissive 4qA allele; they are considered to have Type 2 FSHD (FSHD2, OMIM #158901; <https://www.omim.org/entry/158901>) due to additional mutations involving the structural maintenance of chromosomes flexible hinge domain-containing protein 1 (*SMCHD1*) gene on chromosome 18p11<sup>43</sup> or the DNA methyltransferase 3B (*DNMT3B*) gene on chromosome 20q11.<sup>44</sup> Both types of mutations are associated with insufficient epigenetic repression of D4Z4 repeats leading to aberrant expression of double homeobox protein 4 (*DUX4*) in skeletal muscles and subsequently disease progression.<sup>3</sup> While initially considered as “junk” DNA, each D4Z4 repeat contains an open reading frame for the *DUX4* gene. *DUX4* belongs to a family of genes encoding double homeodomain proteins.<sup>45</sup> A homologue of *DUX4*, known as *DUX4c*, is located distally nearby *DUX4*. The two genes share the same DNA binding homeodomain. However, *DUX4c* lacks the C-terminus transactivation domain and is not cytotoxic when expressed in muscle cells. Previous studies suggested that *DUX4c* may play important roles in muscle regeneration, while *DUX4* expression adversely affects muscle cell survival and differentiation.<sup>46–50</sup> *DUX4* is a nuclear transcription factor that is normally expressed in germ cells.<sup>51,52</sup> Abnormal *DUX4* expression leads to dysregulation of molecular pathways that are involved in muscle differentiation, oxidative stress responses, immune responses, and protein turnover.<sup>51–53</sup> A recent study showed cytoplasmic localization of *DUX4* during muscle differentiation in culture; however, its function in the cytoplasm is not clear.<sup>54</sup> The precise mechanism of how changes in these pathways cause FSHD remains to be elucidated.

Based on recent findings, it is believed that a combination of two genomic features is required to result in FSHD. The first feature is a mutation that causes derepression of *DUX4*

gene, which allows for the *DUX4* transcripts to be transcribed. The second genomic feature is a functional polyadenylation signal in the pLAM region distal to the D4Z4 repeat array, which is associated with the FSHD-permissive 4qA haplotypes.<sup>43,55</sup> Without the polyadenylation signal, the *DUX4* transcripts are quickly degraded. However, when the transcripts from the last D4Z4 repeat are polyadenylated, it stabilizes the *DUX4* mRNA and allows for the translation of the *DUX4* protein (see ►Fig. 2).

In FSHD1, shortening of the D4Z4 repeat array destabilizes the chromatin structure in the 4q35 region, resulting in the transcriptional derepression of the *DUX4* gene. In many patients with FSHD2, mutations in the *SMCHD1* gene lead to haploinsufficiency of *SMCHD1*, which derepresses the D4Z4 region and allows for *DUX4* expression in the presence of a permissive 4qA allele.<sup>43</sup> Recent reports showed that *SMCHD1*, a member of the condensin/cohesin family of chromatin compaction complexes, is also a genetic modifier of FSHD1. Patients with FSHD1 developed more severe disease phenotypes when they carry mutations in *SMCHD1* in addition to contraction of the D4Z4 repeat array.<sup>56,57</sup> In addition to *SMCHD1*, mutations in the *DNMT3B* gene have also been found to modify disease severity and may be responsible for some cases of FSHD2.<sup>44</sup>

## Genetic Diagnosis

According to a recent European Neuromuscular Centre (ENMC) workshop, conventional Southern blot-based methods remain the key diagnostic tools for FSHD; the authors further emphasized the importance of having detailed clinical description at the time of referral for gene testing.<sup>58</sup> Currently, commercial genetic testing for FSHD is limited to FSHD1. Exclusion criteria for FSHD include evidence of an alternative diagnosis based on atypical clinical features, muscle biopsy, or other available investigations.

For individuals with clinical features of FSHD, the diagnosis can be confirmed by determining the size of the D4Z4 repeat on chromosome 4q using Southern blot or molecular combing methods.<sup>58</sup> Tawil et al confirmed that finding a D4Z4 contraction on chromosome 4q35 is highly sensitive (93%) and specific (98%) for the diagnosis of clinically defined FSHD; the degree of specificity is unlikely to be further enhanced in typical cases by testing for the 4qA variant.<sup>38</sup>

Additional genetic analysis or referral to a more specialized laboratory for further DNA tests (such as methylation analysis or FSHD gene panel) may be required if the standard FSHD test is negative. Determining the occurrence of a D4Z4 contraction on a 4qA haplotype is warranted for atypical FSHD, especially in the absence of a first-degree relative with a genetically confirmed diagnosis.<sup>38</sup> Methylation analysis using methylation-sensitive restriction enzymes<sup>56</sup> or bisul-fite sequencing of one or more D4Z4/pLAM sequences<sup>59</sup> can be diagnostic for the presence of pathologic variants in D4Z4-chromatin modifiers. Additional genetic testing for *SMCHD1* or *DNMT3B* may be indicated based on correlation with D4Z4 methylation status.<sup>44</sup>

## Standard of Care for FSHD

Beyond confirming the diagnosis, a recent publication on standard of care offered guidelines on comprehensive assessment and treatment of complications related to FSHD.<sup>38,60</sup>

Important lifestyle strategies include regular physical activities, well-balanced diet, and management of musculoskeletal pain that can be offered to children and adults with FSHD after confirmation of the diagnosis. Scapular fixation in centers experienced with FSHD is potentially safe and effective for carefully selected patients, once they have achieved skeletal maturity. Other anticipatory care includes screening for extramuscular manifestations, particularly among children with early-onset disease.

## Extramuscular Manifestations in Early-Onset FSHD

### Retinal Vasculopathy

Small (1968) first described the association of bilateral retinal exudative telangiectasia, neurosensory deafness, and mental retardation among four siblings with FSHD.<sup>61</sup> A Dutch population survey subsequently found that 3 out of 256 (1.7%) patients with FSHD had retinal vasculopathy with features resembling Coats' disease.<sup>7</sup> More recently, Statland et al estimated the prevalence of retinal vasculopathy in FSHD1 to be at 0.8% (95% CI: 0.2–2.2%); there was a negative association between D4Z4 repeat number and the risk of Coats' disease.<sup>33</sup> Based on a systematic review, Tawil et al concluded that although up to 25% (95% CI: 20.9–30.8%) of patients with FSHD had abnormalities on retinal examination, only 0.6% (95% CI: 0.2–1.5%) had symptomatic retinal disease.<sup>38</sup>

To ensure optimal vision, children with FSHD, especially those with large deletions (10–20 kb or less than five D4Z4 repeats) should be referred to an experienced ophthalmologist for dilated indirect ophthalmoscopy. Subsequent monitoring will depend on the degree of retinal vasculopathy at initial screening. Also, children with unspecified retinal vasculopathy should have careful neurological evaluation to exclude early-onset FSHD.

### Neurosensory Hearing Loss

Lutz et al found that hearing loss preferentially affects individuals with large deletions (<20 kb); one-third (32%; 95% CI: 16.7–51.4%) of their patients in this group had hearing loss and some were progressive.<sup>35</sup> Tawil et al concluded in their systematic review that although 15.5% (95% CI: 12.1–19.4%) of patients with FSHD had evidence of audiometric abnormalities, the estimate was hampered by the significant variability in the frequencies reported in the literature.<sup>38</sup>

The current guideline therefore recommends that young children with FSHD should have hearing assessment at diagnosis and yearly thereafter until entry into the school. Older children do not need formal evaluation if their language development is normal and hearing is routinely monitored in school. Similarly, adults with FSHD generally do not require audiograms unless they are symptomatic.

### Central Nervous System Involvement

Rarely, FSHD can be associated with cognitive disability and epilepsy in childhood, particularly among those with very few D4Z4 repeats.<sup>62–66</sup> In line with previous studies, Chen et al found that epilepsy frequently occurred with mental retardation among patients with very short EcoRI fragments (10–12 kb or less than three D4Z4 repeats).<sup>30</sup> Given the risk of epilepsy in these individuals, education regarding seizure recognition and epilepsy safety should be offered at diagnosis; a sleep-deprived electroencephalogram (EEG) may be indicated for those with suspected seizures. Also, facial weakness and auditory or visual disturbance could be obscured by the presence of profound intellectual disability in early-onset FSHD.<sup>67</sup> Formal visual and auditory assessments on a regular basis plus timely intervention are required to ensure optimal developmental outcomes for these children.

### Cardiac and Respiratory Involvement

Respiratory insufficiency and cardiomyopathy are relatively uncommon in FSHD. Routine cardiac screening is not required in the absence of any cardiac symptoms. However, given that children may not be able to express their symptoms, an initial echocardiogram and electrocardiogram (ECG) assessment is reasonable for those diagnosed with early-onset disease. Similarly, a baseline pulmonary function test is recommended for all patients with FSHD, especially those with severe involvement. In a recent pediatric study, respiratory volumes were found to correlate with disease severity, and the expiratory phase appeared to be specifically affected in early-onset FSHD compared with other muscular dystrophies.<sup>68</sup> Those with abnormal baseline pulmonary function, significant weakness, or other comorbid conditions that may affect ventilation should be monitored regularly or referred to a specialist in pulmonary and sleep medicine for consideration of overnight sleep studies or noninvasive positive-pressure ventilation.

### Clinical Evaluations of FSHD

Accurate assessments are essential for clinical care and research; these can provide information regarding the extent of functional impairment and detect potential response to disease-modifying treatments. Common clinical evaluations for FSHD include quantitative muscle strength testing (QMT), timed function test, and clinical severity scores.<sup>38</sup> The use of age-appropriate motivating audiovisual feedback such as the Cooperative International Neuromuscular Research Group quantitative muscle testing (QMT) system can enable maximal isometric contraction and reliable muscle testing in young children.<sup>69</sup> Recently, Eichinger et al described the use of timed function tests, including the 6 minute walk test (6MWT), the Timed Up and Go (TUG), and the 30 foot Go/Timed 10 m test, as measures of mobility among participants with FSHD.<sup>70</sup> They found the 6MWT to be reliable and consistent when compared across two study sites using standardized protocols. The 6MWT also showed moderate to strong correlations with other assessments of FSHD disease severity, including measures of strength and function. These timed function tests are likely to be included as outcome measures for future natural history studies and clinical trials for FSHD.

Lamperti et al developed a clinical evaluation scale (CES)<sup>71</sup> to enable functional quantification of muscle weakness for patients with FSHD. The sum of six regional (facial, scapular, upper limb, distal leg, pelvic girdle, and abdominal) muscle groups generates a FSHD clinical severity score with total scores ranging from 0 to 15. The score can be further grouped into grades of disease involvement, for example, 0 (unaffected), 1–2 (mild), 3–6 (moderate), or 7–15 (severe). More recently, Ricci et al revised the FSHD clinical form to expand the phenotypic spectrum as observed in FSHD.<sup>72</sup> The new comprehensive clinical evaluation form (CCEF) allows for precise categorization of patients; furthermore, it may potentially be useful for genetic counseling, selection of subjects in treatment trials, as well as research into other genetic factors contributing to the clinical variability in FSHD.<sup>72</sup> Both the FSHD CES and the CCEF can be adapted for assessment of pediatric-onset FSHD.

## Additional Evaluations

### Muscle Magnetic Resonance Imaging

Muscle MRI is increasingly used to identify specific pattern of involvement in different types of muscular dystrophies. It is particularly useful in detecting selective muscle involvement of nonclinically testable muscles; furthermore, MRI can potentially be used as outcome measures in clinical trials as well as longitudinal studies of FSHD.<sup>73,74</sup> However, young children or those with cognitive disability may require sedation or general anesthesia to undergo a formal muscle MRI study.

Recently, Gerevini et al detected a specific pattern of muscle fatty replacement and atrophy, particularly in upper girdle muscles using MRI. According to Gerevini et al, the most frequently affected muscles, including paucisymptomatic and severely affected FSHD patients, were the trapezius, teres major, and serratus anterior; moreover, asymmetric muscle involvement was significantly higher in FSHD as compared with non-FSHD patients.<sup>74</sup> In another study, Tasca et al reported that the combined involvement of abdominal and hamstring muscles with iliopsoas sparing was relatively common in FSHD (67% of the patients). They also found that the extent of lesions on lower limb MRI showed a high correlation with overall clinical severity, with one-fourth of their nonpenetrant gene carriers showed abnormalities on MRI.<sup>73</sup> Furthermore, potential markers of active disease, as indicated by hyperintensities on short-tau inversion recovery sequences, were found in muscles without signs of fatty replacement in one-third of their patients. The authors suggested that the hyperintensities were indicative of early stages of muscle involvement in FSHD.<sup>73</sup>

### Biomarker Exploration for FSHD

Biomarkers that can be used to evaluate the effects of treatment in much more acute time frames are highly desirable, especially those that are quantitative in nature and not subjected to effort or bias. Surrogate biomarkers available from a less invasive procedure such as blood collection (instead of muscle biopsies) will be a valuable outcome measure for evaluating therapeutic efficacy in clinical studies for FSHD, especially in children with significant muscle weakness. Previous studies identified both protein and miRNA biomarker candidates in patients' sera.<sup>75,76</sup> Recently, Petek et al utilized the sensitive, specific, high-throughput

SomaLogic proteomics platform of 1,129 proteins to identify proteins with levels that correlated with FSHD severity. They reported that the levels of creatine kinase (both MM and MB isoforms), carbonic anhydrase III, and troponin I type 2 were found to reliably predict the disease state and correlated with disease severity.<sup>77</sup> Moreover, the novel biomarkers may help reveal other mechanisms of disease in FSHD. Further confirmatory studies are pending.

## New Emerging Treatments for FSHD

Although there is presently no effective pharmacological treatment, recent discoveries have improved our understanding on the pathogenesis of FSHD and allowed researchers to focus their efforts on epigenetic and transcriptional regulation of the aberrantly expressed *DUX4* in FSHD. *DUX4* is a retro-transposed gene with several alternative splice variants.<sup>78</sup> Snider et al reported that the full-length splice variants of *DUX4* (*DUX4-fl*) are aberrantly expressed in FSHD muscle cells but not in the muscles of healthy individuals. In addition, expression of the *DUX4-fl* transcript is restricted to testis and possibly germline cells in healthy individuals. Several studies showed that *DUX4* upregulated germline genes in FSHD muscle cells when ectopically expressed in muscle cells.<sup>53,79,80</sup> *DUX4* is toxic independent of tissue types when it is expressed ectopically in frogs, zebra fish, and mice.<sup>81,82</sup> In addition, it is proapoptotic when aberrantly expressed in mouse and human muscle cells.<sup>51,81,83</sup> Additional molecular pathways misregulated by *DUX4* include oxidative stress responses,<sup>84,85</sup> immune responses,<sup>52</sup> transcription regulation,<sup>54,86,87</sup> protein turnover,<sup>88,89</sup> and myogenesis program.<sup>53</sup> Currently, therapeutic strategies targeting different steps in the cascade of *DUX4* regulation are under investigation and include (a) restoring epigenetic state at the D4Z4 region, (b) knocking down the aberrantly expressed *DUX4* (c) reducing the cytotoxic effects downstream of *DUX4*, and (d) counteracting the pathological changes by activating compensatory pathways. The following are examples of treatment strategies that have been advanced into preclinical or clinical studies for FSHD.

### Oligonucleotide-Based Gene Therapy

In the past three decades, different oligonucleotide-based gene silencing strategies have been developed to target specific genes for treating infectious and genetic diseases. Some of these gene-silencing strategies are being evaluated in clinical trials and two of these are United States Food and Drug Administration (FDA) approved drugs (eteplirsen and nusinersen) for neuromuscular diseases. Different modifications of existing strategies were developed and investigated to overcome common problems associated with these gene-silencing strategies, including (a) poor stability, (b) inefficient delivery into target cells, (c) off-target/toxicity effects, and (d) immunogenicity.<sup>90,91</sup> Several backbone modifications of these oligonucleotides improve their stability as RNA-silencing agents. Protein peptides or other molecules are conjugated to the oligonucleotides to increase delivery efficiency. However, such modifications often increased toxicity and/or immunogenicity. To date, oligonucleotide strategies are still evolving quickly, and they will require further preclinical studies.

For FSHD, several approaches have been developed to sequester or degrade the target mRNAs, including shRNA, miRNA, siRNA, and antisense oligonucleotides (AON).<sup>92-95</sup>



Before *DUX4* was considered as the causative gene of FSHD, candidate genes in the 4q35 region were investigated for their potential involvement in FSHD. These were the facioscapulohumeral muscular dystrophy region gene 1 (*FRG1*), facioscapulohumeral muscular dystrophy region gene 2 (*FRG2*), and adenine nucleotide trans-locator 1 (*ANT1*).<sup>96</sup> The shRNA and miRNA against *FRG1* were delivered using adeno-associated viral (AAV) vector, which successfully reduced the *FRG1* expression in vivo.<sup>93,94</sup> Later, miRNA against *DUX4* was delivered by AAV vector into mouse muscles ectopically expressing *DUX4* and was able to reduce *DUX4* and improve pathologies induced by it.<sup>95</sup> In addition, morpholinos were shown to either successfully reduce *DUX4* expression or inhibit translation of a *DUX4*-regulated gene, termed paired-like homeodomain transcription factor 1 (*Pitx1*).<sup>89,90,98</sup> One major challenge of AON therapy is the limited uptake by muscle in vivo. Currently, several new compounds with gymnosin property are under investigation.

### Reduce Muscle Inflammation by Immune Modulatory Agents

Previous studies reported that severe inflammatory responses were found in approximately 30% of patients with FSHD. Atypical inflammatory responses, including activation of both innate and acquired immune responses, were shown in FSHD muscles, although autoantibodies that are commonly detected in myositis were not detected in FSHD.<sup>79,98,99</sup> In addition, molecular studies reported activation of immune-related genes when *DUX4* is activated in muscle cells.<sup>51,52</sup> These findings suggested active roles of immune system in disease mechanisms of FSHD. Pharmaceutical agents that modulate immune responses may potentially benefit the disease. A pilot trial of prednisone was conducted and showed no significant effect, although this may have been due to the short trial period to detect significant improvement or delay in disease progression.<sup>100</sup> Currently, clinical trials testing an immune modulatory compound, ATYR1940, are being conducted by ATyr Pharma Inc. The compound is a physiocrine-based protein (histidyl tRNA synthetase) with multiple functions and is believed to modulate immune responses in skeletal muscles.<sup>101,102</sup>

### Improve Muscle Mass and Pathology by Activating Compensatory Pathways

Previous studies showed that aberrant expression of *DUX4* led to activation of molecular pathways involved in skeletal muscle atrophy.<sup>89</sup> One potential therapeutic strategy will be to activate compensatory pathways that will counteract the muscle atrophy induced by *DUX4*. Myostatin is a member of the transforming growth factor- $\beta$  superfamily. It negatively regulates muscle mass during fetal development and in postnatal muscles.<sup>103</sup> Several species with mutated myostatin have hypertrophic muscles, supporting the role of myostatin as a negative regulator of muscle mass in the whole body. Myostatin is downregulated during normal muscle regeneration and in muscular dystrophies.<sup>104–108</sup> Several animal studies have demonstrated beneficial effects of myostatin inhibitors<sup>109–113</sup> although this has not been tested in a FSHD model. A clinical study examining safety of a neutralizing antibody to myostatin, MYO-029, was conducted and concluded that the agent was relatively safe.<sup>114</sup> Clinical studies on locally-acting myostatin inhibitor, ACE-083, are currently conducted by Acceleron Pharma.

## Other Treatments

Other potential treatments include exercise therapy, cell therapy, as well as studies of compounds that target molecular pathways and are misregulated by *DUX4*. The latter includes a clinically approved tyrosine kinase inhibitor (sunitinib),<sup>115</sup> poly ADP ribose polymerase (PARP1) inhibitors,<sup>116</sup> and antioxidants.<sup>117</sup> FSHD cells have been reported to be more susceptible to oxidative stress. A clinical study with vitamin C, vitamin E, zinc, and selenium supplements indicated potential benefits to the disease.<sup>118</sup> Exercise and behavioral intervention have also shown beneficial effects for individuals with FSHD.<sup>119–121</sup> Several clinical trials testing different regimens of exercises are currently ongoing.

A recent study investigated the fusion of FSHD myoblasts with primary myoblasts from healthy individuals to correct differentiation defects in culture.<sup>122</sup> The authors concluded that more than 50% of normal myoblasts were required to correct the phenotypical and functional defects observed in FSHD; therefore, cell therapy in the FSHD is potentially feasible but with limitations. In addition to genome editing, a CRISPR/dCas9 transcriptional inhibitor was targeted to the D4Z4 array to silence the aberrantly expressed *DUX4*.<sup>123</sup> The study demonstrated the potential of using CRISPR/dCas9 transcriptional inhibitor to correct the epigenetic defects in FSHD.

## Conclusion

FSHD is a heterogeneous disease; children with early-onset disease are at a risk of more rapid decline in motor function, early loss of independent ambulation, as well as extramuscular manifestations. The elucidation of a unifying model for FSHD has provided several potential therapeutic approaches to treat FSHD. Early diagnosis, ongoing support as per standard of care guidelines, and collaboration with global disease registries will be essential for optimizing the health outcome of affected individuals, as well as clinical trial readiness for FSHD.

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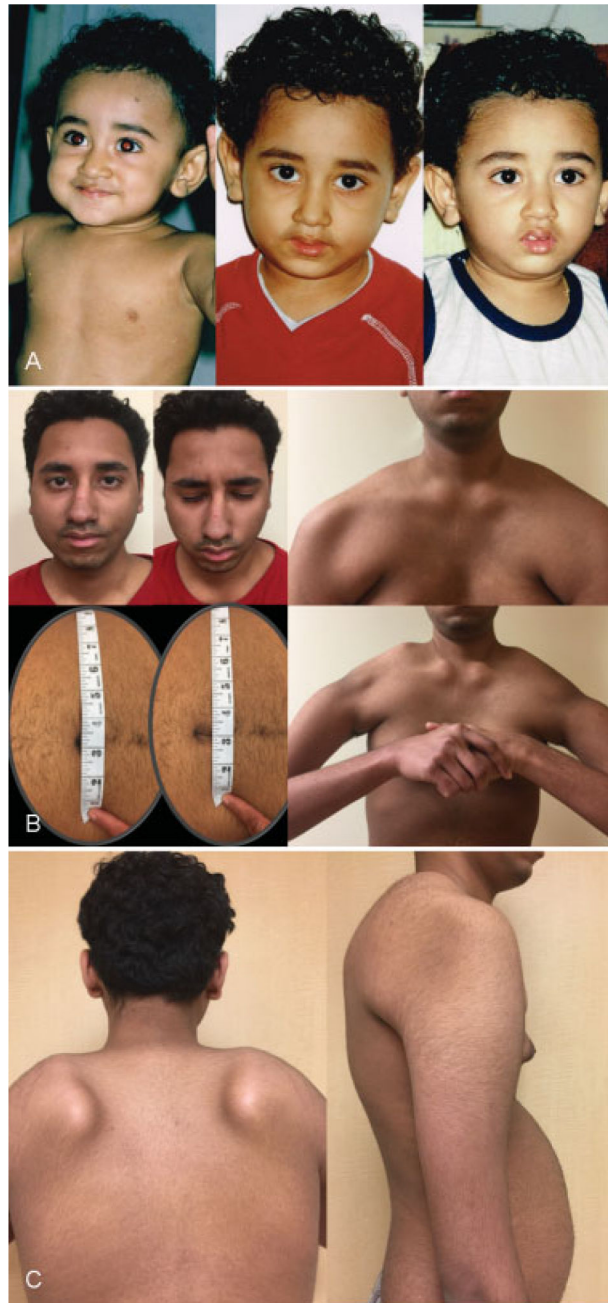
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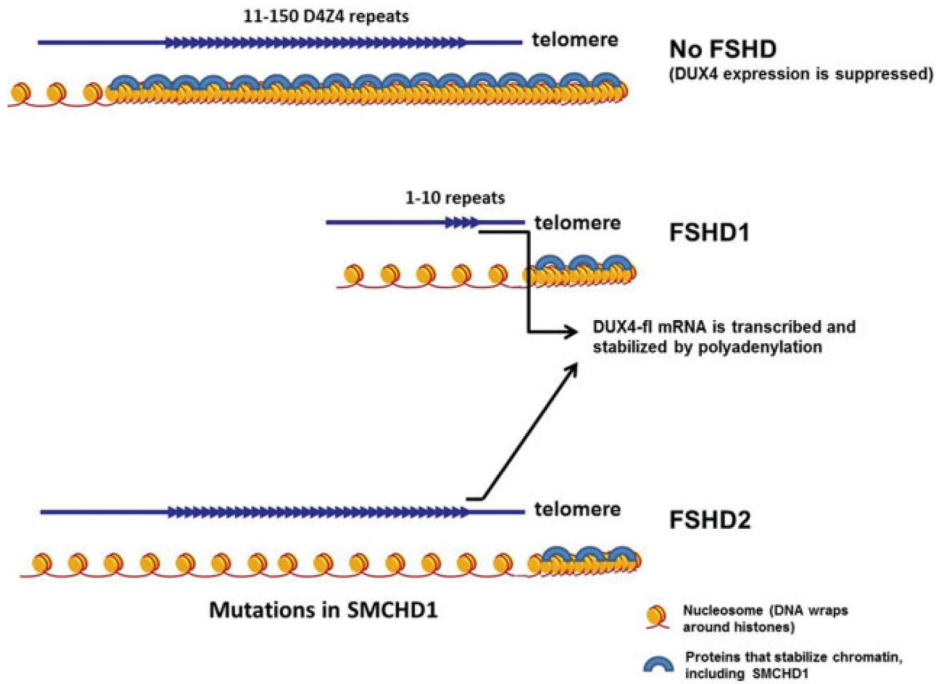
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**Fig. 1.** Clinical features of early-onset FSHD. (A) Evidence of mild asymmetrical facial weakness with onset after 1 year of age (right panel). (B) Clinical features of FSHD at 16 years of age, including eye closure weakness (left upper panel), Beevor's sign (left lower panel; the umbilicus moved upward with neck flexion), pectus excavatum (right upper panel), high-riding scapula, horizontal clavicles, and poly-hill sign due to prominent periscapular muscle atrophy (right lower panel). (C) Scapular winging (left panel) and lumbar lordosis (right panel). FSHD, facioscapulohumeral muscular dystrophy.



**Fig. 2.** Genetic mechanisms of FSHD. Two genomic features are required to cause FSHD. The first is a contraction of the D4Z4 array from 11 to 150 repeats to 1 to 10 repeats in patients with FSHD1 and mutations in *SMCHD1* in patients with FSHD2. Both loosen chromatin structure of the D4Z4 region and allow transcription of *DUX4*. The second feature is a functional polyadenylation signal (PAS) downstream of the last D4Z4 repeat, which allows the *DUX4* transcript to be stabilized for protein translation. FSHD, facioscapulohumeral muscular dystrophy.