Facioscapulohumeral Muscular Dystrophy

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REVIEW ARTICLE

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ABSTRACT

PURPOSE OF REVIEW: **This article reviews the current knowledge on the clinical characteristics and disease mechanism of facioscapulohumeral muscular dystrophy (FSHD), as well as advances in targeted therapy development.**

RECENT FINDINGS: **FSHD has a wide range of severity, yet a distinct phenotype characterized by weakness of the facial, shoulder, and upper arm muscles, followed by weakness of the trunk and leg muscles. It can be caused by two genetic mechanisms that share a common downstream pathway, namely, the epigenetic derepression and subsequent misexpression of the myotoxic *DUX4* transcription factor. Treatment is currently supportive and outlined in evidence-based guidelines. Advances in the understanding of the pathogenic mechanism of FSHD are paving the way for targeted therapy development. Approaches for targeted therapies to reduce *DUX4* expression that are currently being explored include small molecules, antisense oligonucleotides, vector-based RNA interference, and gene therapy. In anticipation of more clinical trials, "clinical trial preparedness," including the development of sensitive biomarkers and clinical outcome measures, are needed.**

SUMMARY: **The cornerstones of the diagnosis of FSHD are clinical observation and genetic testing. Management is currently supportive, but progress in the understanding of the disease mechanism has shifted the field of FSHD toward targeted therapy development.**

INTRODUCTION

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent inherited muscle disorders and occurs in 5 to 12 per 100,000 individuals.[1] In 1885, Landouzy and Dejerine reported a distinct phenotype consisting of facial weakness and progressive muscle weakness, and wasting in a descending course along the limbs and trunk.[2] Since then, the clinical picture of this disease, which is now known as FSHD, has been further refined. Molecular studies have revealed that FSHD can be caused by two different genetic pathways that converge on one common downstream mechanism.[3] While the treatment of FSHD is currently only supportive, advances in the understanding of the disease mechanism have paved the way for the development of targeted therapies. To be able to effectively test newly developed therapies, effort is now being put into the preparation of future clinical trials.[4]

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This article provides an overview of the clinical features of FSHD, diagnostic considerations, and current treatment. In addition, the two genetic mechanisms and progress in therapy development are discussed.

CLINICAL PICTURE

FSHD has a characteristic pattern of muscle involvement, often starting with asymmetric weakness and atrophy of muscles of the face, shoulder girdle, and

CASE 9-1

A 16-year-old girl presented to the outpatient clinic with right-sided shoulder complaints that began after she accidentally dropped a heavy bag, thereby overstretching her shoulder. In the days after the incident, she noticed trouble lifting her right arm. She did not experience pain.

On neurologic examination, bilateral scapular winging was noticed, most pronounced on the right side. She was able to lift her right arm to 90 degrees and her left arm to 120 degrees. Atrophy of the right major pectoral muscle and a horizontal axillary fold was observed. She had wide-open eyes and an asymmetric mouth. Examination of her face revealed a *signe de cils* (the inability to bury the eyelashes completely when closing the eyes tightly) and asymmetric pouting of the lips (FIGURE 9-2).

Facioscapulohumeral muscular dystrophy (FSHD) was suspected and genetic testing revealed a D4Z4 fragment size of 25 kb, confirming a diagnosis of FSHD type 1. After this diagnosis, her parents were examined. Her 46-year-old mother had isolated facial weakness and indeed carried the shortened D4Z4 fragment.

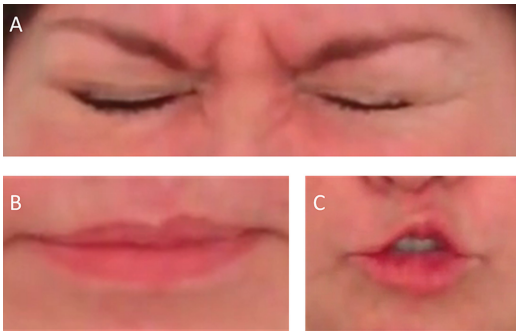

FIGURE 9-2

Facial weakness in the patient in CASE 9-1. *A*, Bilateral *signe de cils*, an inability to bury the eyelashes completely when closing the eyes tightly, most pronounced on the right side. *B*, Asymmetric lips in resting position. *C*, Asymmetric pouting of the lips (right-sided weakness).

COMMENT

Patients with FSHD can present in many ways that may not directly trigger the search for an inherited muscle disorder, especially those without a positive family history. Initial presentations include, among others, bent spine syndrome, (unilateral) limb weakness such as footdrop, frequent falling, and a variety of shoulder complaints. In this case, an observant neurologist recognized mild signs of facial weakness, leading to a swift diagnosis. Many patients with FSHD are not aware of their facial weakness and rarely spontaneously report this as a symptom. Furthermore, this case illustrates the large variability in symptom onset and disease course between members of the same family.

upper arms.[5] In later disease stages, the trunk, pelvic girdle, and leg muscles often become affected as well. The onset of muscle weakness is typically between ages 15 to 30 years, although this varies from infancy to late adulthood. Disease severity varies substantially, even within families. The severity spectrum ranges from asymptomatic gene carriers who only show minor signs of the disease on physical examination, to severe generalized weakness. Muscle weakness is slowly progressive over the lifetime, although individual patients often report periods of rapid deterioration of selective muscles followed by periods of stabilization of disease activity. Life expectancy is generally not reduced, but morbidity can be significant; approximately 20% of patients eventually become wheelchair dependent.[6]

In most cases, an autosomal dominant pattern of inheritance is present. However, family history can be negative as 10% to 30% of cases are caused by de novo mutations and a rarer genetic form of FSHD (FSHD2) has a digenic mode of inheritance (see the Disease Mechanism section).[7,8] Additionally, nonpenetrant and minimally affected gene carriers can be found in up to 30% of family members in certain families.[9]

In approximately 10% of patients, the disease manifests before age 10. This subgroup of so-called "infantile cases" has a more severe disease course, with 40% becoming wheelchair dependent during childhood.[10] Extramuscular manifestations of FSHD are rare but can include retinal vasculopathy (sometimes progressing to Coats syndrome), sensorineural hearing loss, restrictive lung disease, and (incomplete) right bundle branch block, although all are mostly subclinical. Cardiomyopathy and involvement of respiratory muscles are generally not associated with FSHD. Infantile cases are most at risk for extramuscular involvement.

CLINICAL CLUES IN THE EXAMINATION ROOM

Although FSHD has a highly characteristic phenotype, specific clinical signs can be subtle, especially in early disease stages or mild cases. FSHD can be diagnosed based on clinical observations and confirmation by DNA testing. Therefore, it is important for neurologists to recognize this disorder and prevent unnecessary diagnostic delay (CASE 9-1). To facilitate swift recognition, the following overview provides symptoms and signs that may be suggestive of, or sometimes almost pathognomonic for, FSHD (FIGURE 9-1).

Face

Facial muscle weakness is one of the first signs of FSHD.[5] Patients rarely report symptoms of facial weakness spontaneously, and it is easily overlooked by physicians on examination. Most commonly affected are the circular muscles around the eyes (orbicularis oculi) and mouth (orbicularis oris).[11] Physicians should actively ask for symptoms of facial weakness such as sleeping with the eyes (partially) opened or an inability to pucker, whistle, or drink from a straw. Difficulty in raising the corners of the mouth due to weakness of the zygomaticus major muscle results in the mouth moving horizontally on attempts to smile, causing a so-called "transverse smile." Facial weakness can be very subtle in approximately 10% of patients.[11] Due to the change in facial expression, patients may be falsely perceived as arrogant or grumpy. Ptosis and involvement of the extraocular muscles do not occur in FSHD.

KEY POINTS

- Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common forms of muscular dystrophy with a prevalence of 5 to 12 per 100,000 individuals.
- FSHD has a highly characteristic phenotype that starts with asymmetric weakness of the facial, shoulder, and upper arm muscles, and later of the trunk and leg muscles. Severity, age at onset, and rate of progression of muscle weakness vary greatly.
- In most cases of FSHD, a positive family history is present. However, family history can be negative due to de novo mutations, incomplete penetrance, or a genetic form of FSHD with a digenic inheritance pattern (FSHD2).
- The infantile form of FSHD is characterized by an early disease onset (before age 10 years) with generalized and rapidly progressive muscle weakness and a higher chance of extramuscular complications.
- Extramuscular disease manifestations in FSHD are mostly subclinical and can include retinal vasculopathy, sensorineural hearing loss, restrictive lung disease, and (incomplete) right bundle branch block. Cardiomyopathy is not associated with FSHD.

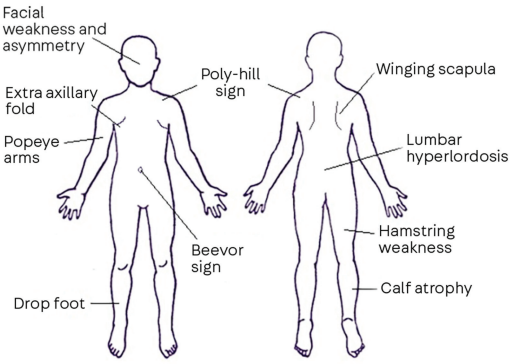

FIGURE 9-1
Characteristic signs of facioscapulohumeral muscular dystrophy.
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Shoulders and Upper Arms

Another highly characteristic sign of FSHD is asymmetric scapular winging, which is due to weakness of the scapular fixator muscles, in particular, the serratus anterior and trapezius muscles. Patients with FSHD are often unable to lift their arms to 180 degrees. The humeral muscles (biceps and triceps) are typically wasted with sparing of the forearm muscles, resulting in "Popeye" arms. The supraspinatus and infraspinatus muscles are typically spared.[12,13]

The selective wasting of muscles can result in the typical "poly-hill" sign (FIGURE 9-3).[14] The "hills" consist of (1) a combination of atrophy of the trapezius muscle and upward movement of the scapula, (2) displacement of the acromioclavicular joint, and (3) a combination of wasting of the proximal part of the deltoid muscle and biceps and sparing of the distal part of the deltoid muscle.

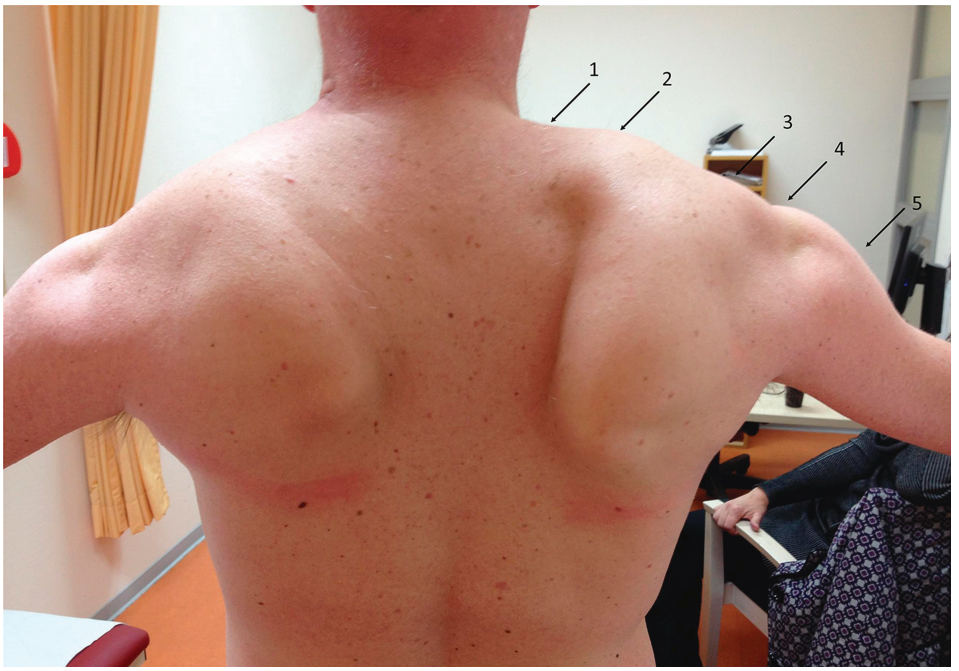

FIGURE 9-3
Scapular winging and poly-hill sign. Pronounced bilateral, but asymmetric, scapular winging. Note the typical poly-hill configuration on the right shoulder caused by (1) atrophy of the trapezius muscle, (2) superior angle of the overriding scapula, (3) displaced acromioclavicular joint, (4) atrophy of the proximal deltoid muscle, and (5) sparing of the distal deltoid muscle.

Trunk

An often underappreciated symptom of FSHD is the involvement of the abdominal muscles, which can cause difficulty rising from a supine to a sitting position, a protruding abdomen, and lumbar hyperlordosis. Often predominant involvement of the lower abdominal muscles exists, which causes Beevor sign, which is an upward movement of the umbilicus on flexing the neck in the supine position.[15]

Involvement of the major pectoral muscle is common and results in a horizontal axillary fold. Many patients have weakness of the paraspinal muscles that, when severe, can lead to bent spine syndrome.[16]

Legs

Although the name of the disease suggests otherwise, most patients with FSHD have at least some degree of lower extremity weakness, especially in later disease stages. Clinically, the first manifestation of leg muscle involvement is often the occurrence of foot drop. However, muscle imaging studies have revealed that involvement of other leg muscles starts before weakness is noticed.[13,17] This includes subclinical involvement of the hamstring, adductor, rectus femoris, and medial gastrocnemius muscles. Because these muscles function as part of a muscle group, functionality is probably preserved by compensation of other muscles of the same group.

DISEASE MECHANISM

Two genetic forms of FSHD exist, FSHD type 1 (FSHD1) and FSHD type 2 (FSHD2), that share a similar clinical presentation.[8,18] In both FSHD1 and FSHD2 muscle cell death is caused by a common downstream mechanism, namely, the misexpression of the double homeobox 4 (*DUX4*) gene, which is normally epigenetically silenced in most somatic tissues (FIGURE 9-4[19]).

A copy of the *DUX4* gene is embedded within each unit of the D4Z4 macrosatellite repeat array on chromosome 4q35.[18] This D4Z4 repeat array varies between 8 to 100 repeat units in the healthy population. In FSHD1, the most common form of FSHD accounting for approximately 95% of patients, the size of this D4Z4 repeat array is reduced to 1 to 10 repeat units. This repeat contraction results in a more open chromatin structure of the repeat array in somatic cells, allowing expression of the *DUX4* gene. However, for the DUX4 transcript to be stable, a polyadenylation signal is required that is only present on a 4qA disease-permissive haplotype distal to the D4Z4 repeat. A D4Z4 repeat contraction on the equally common 4qB haplotype does not cause FSHD.

Approximately 5% of all patients with FSHD display D4Z4 chromatin relaxation and somatic derepression of *DUX4* without having a D4Z4 repeat contraction.[8] These patients with FSHD2 typically carry D4Z4 repeat arrays of 11 to 30 repeat units, which is shorter than the general population. In FSHD2, pathogenic variants in chromatin modifier genes cause D4Z4 hypomethylation and thus, a more open chromatin structure of the D4Z4. Over 85% of all FSHD2 cases are caused by heterozygous mutations in the *SMCHD1* (structural maintenance of chromosomes flexible hinge domain containing 1) gene on chromosome 18. Other rare causes that have been identified are heterozygous mutations in the DNA methyltransferase 3 beta (*DNMT3B*) gene and homozygous mutations in the *LRIF1* (ligand-dependent nuclear receptor-interacting factor 1) gene.[20,21] Just as in FSHD1, a permissive 4qA haplotype is required to stabilize the

KEY POINTS

- Two genetic forms of FSHD, FSHD1 and FSHD2, occur and share a common downstream "gain of function" mechanism, namely, the misexpression of the myotoxic *DUX4* gene. Clinically, the two forms are indistinguishable.
- FSHD1 is caused by a contraction of the D4Z4 repeat array on chromosome 4q35 to 1 to 10 D4Z4 units, resulting in a more open chromatin structure allowing expression of the *DUX4* gene. A permissive polymorphism provides a polyadenylation sequence to stabilize the DUX4 transcript.
- In patients with FSHD2, D4Z4 chromatin relaxation is caused by mutations in chromatin modifier genes (most often *SMCHD1*) in the absence of a repeat contraction. Due to the required polyadenylation sequence on chromosome 4, FSHD2 has a digenic pattern of inheritance.

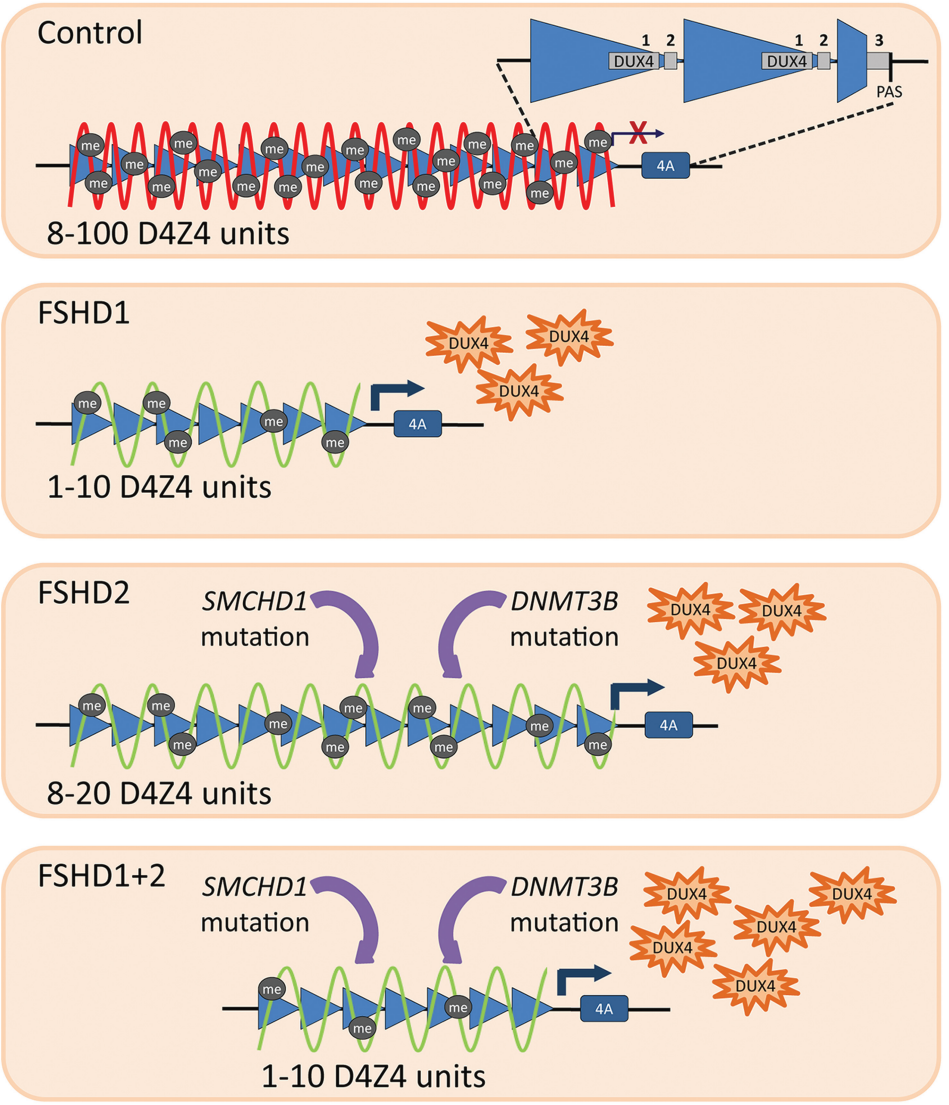

FIGURE 9-4

Genetic mechanisms of facioscapulohumeral muscular dystrophy (FSHD). In controls the D4Z4 repeat array (*triangles*) on chromosome 4q varies between 8 and 100 units and adopts a repressed chromatin structure (*red wavy lines*) characterized by high CpG methylation (me). Each D4Z4 repeat contains a copy of the *DUX4* gene, without a polyadenylation signal (PAS). Only on 4qA chromosomes (and not on 4qB chromosomes) is the last repeat unit followed by a third exon which contains a polyadenylation signal that can stabilize the DUX4 transcript from the last repeat unit. FSHD is related to chromatin relaxation (*green wavy lines*) of the D4Z4 repeat array, facilitating the stable expression of DUX4 from a 4qA chromosome. FSHD type 1 (FSHD1) is caused by a contraction of the D4Z4 repeat array to a size of 1 to 10 units. FSHD type 2 (FSHD2) is caused by a mutation in a chromatin modifier gene, most often *SMCHD1* and sometimes *DNMT3B* or *LRIF1*. Mutations in chromatin modifier genes can also act as modifiers of disease severity in FSHD 1.

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DUX4 transcript. Because of the required combination of a pathogenic variant in a chromatin modifier and a borderline-sized D4Z4 repeat array on a permissive haplotype, FSHD2 has a digenic inheritance pattern (CASE 9-2).

PHENOTYPE-GENOTYPE CORRELATIONS

With current knowledge of the disease mechanism, the large variability in disease severity in FSHD can be partially explained. In FSHD1 a rough inverse correlation exists between the number of residual D4Z4 repeat units and clinical severity, but even among family members with the same number of repeat units, often variability in disease severity is seen.[22–24] Patients with very short repeat array sizes of 1 to 3 units have a higher chance of having an "infantile" phenotype with severe muscle weakness and an increased risk of extramuscular complications.[25,26] On the other hand, patients with 7 to 10 repeat units tend to be more mildly affected, or even have a chance of remaining asymptomatic (no symptoms on history, but signs of muscle weakness on examination) or nonpenetrant (without signs on examination).[9,27]

In addition to causing FSHD2, pathogenic variants in the *SMCHD1* gene have been shown to act as a disease modifier in families where both FSHD1 and FSHD2 mutations occurred (FIGURE 9-4).[28] Family members with both a D4Z4 repeat array size of 8 to 10 units and an *SMCHD1* mutation were more severely affected compared to family members with either of the two mutations.

DIAGNOSTICS AND DIFFERENTIAL DIAGNOSIS

A diagnosis of FSHD should be considered in all patients who present with (mild) facial weakness, scapular winging, and asymmetric weakness of limb muscles.

Although the extent and exact distribution of muscle weakness vary between patients, the phenotype of FSHD is well defined. Any of the following signs or red flags makes FSHD highly unlikely and should warrant investigations into other diagnoses: ptosis or involvement of the extraocular muscles, prominent dysphagia or involvement of the masticatory muscles, contractures, weakness of the distal arm muscles in early disease stages, early respiratory involvement, or cardiomyopathy.

The most important differential diagnostic consideration is limb-girdle muscular dystrophy type 2A (calpainopathy), which can present with pronounced scapular winging. Other disorders that may resemble FSHD include Pompe disease (acid maltase deficiency), mitochondrial myopathies, and inclusion body myositis.

Most ancillary investigations such as electrodiagnostic testing, serum creatine kinase (CK) measurement, and muscle biopsy are of little added value in the diagnosis of FSHD. These tests mainly serve to exclude other conditions. Blood CK levels are normal or mildly elevated (never more than 5 times normal). Similarly, electrodiagnostic testing may be normal or reveal nonspecific myopathic features and sometimes signs of denervation possibly related to regenerating fibers or muscle inflammation. Muscle biopsy findings include nonspecific myopathic changes such as fiber size variability, internal nuclei, fibrosis, regenerating fibers, and fatty replacement. Occasionally, inflammatory infiltrates are found.

GENETIC TESTING

In patients with a classic FSHD phenotype and a first-degree relative with genetically confirmed FSHD1, a clinical diagnosis of FSHD is sufficient. In all other cases where there is a clinical suspicion of FSHD, genetic testing is indicated.

KEY POINTS

- In FSHD1, patients with very short D4Z4 repeat array sizes of 1 to 3 units have a higher risk of a severe phenotype, while patients with 7 to 10 repeat units tend to have a milder disease course and decreased penetrance.
- A diagnosis of FSHD is based on clinical observation and genetic testing. Ancillary investigations such as blood creatine kinase, electrodiagnostic testing, and muscle histology show nonspecific findings and are only useful to exclude other diagnoses.

In most protocols, the size of the D4Z4 repeat contraction on chromosome 4 is assessed first.[29] Patients with FSHD1 carry fragments between 10 kb to 38 kb, while in healthy individuals fragments of greater than 38 kb are found. It is only when the clinical presentation is atypical for FSHD that confirmation of the A haplotype on the contracted 4q allele is required.

If testing for FSHD1 is negative (ie, D4Z4 repeat array is greater than 38 kb), the degree of methylation of the 4q35 subtelomeric region can be assessed. Very low methylation of less than 20% on a 4qA haplotype confirms the diagnosis of FSHD2. In most of these patients a pathogenic variant in a chromatin modifier gene, most often the *SMCHD1* gene, can be found by Sanger sequencing. As FSHD2 is a digenic disease, the presence of an *SMCHD1* mutation alone is not sufficient to confirm the diagnosis.

CASE 9-2

A 30-year-old man presented with slowly progressive limb muscle weakness. He had never been able to lift his arms above his head. He tripped regularly due to difficulty lifting his left foot and fell a few times in the last year. Over the last few months, he noticed more rapid progression of weakness and wasting of his right upper arm muscles. No family members reported similar concerns.

On neurologic examination, he had difficulty closing his eyes and a transverse smile. Prominent scapular winging with a poly-hill sign was present, and he could only lift his arms to 90 degrees. His right biceps was atrophied and weak, with preserved muscle bulk and strength of the forearm muscles (Popeye arms). He was unable to rise from a supine to a sitting position without the use of his arms. Mild weakness of the hamstring muscles of the left leg, and more pronounced weakness of the ankle dorsiflexors on the left was present.

Because of the high clinical suspicion for facioscapulohumeral muscular dystrophy (FSHD), genetic testing was performed, which showed a 50 kb D4Z4 fragment size (approximately 14 units), inconsistent with a diagnosis of FSHD type 1 (FSHD1). Next, methylation analysis was performed and revealed a reduced degree of methylation of 15%. Sanger sequencing identified the presence of a pathogenic variant in the *SMCHD1* gene, thereby confirming a diagnosis of FSHD type 2 (FSHD2). Additional genetic testing of family members showed that the patient had inherited the 14-unit D4Z4 repeat array on a permissive 4qA allele from his father, and the *SMCHD1* pathogenic variant from his mother, who were both clinically unaffected (FIGURE 9-5).

Of note is that FSHD1, being caused by a repeat contraction, is not detected by high-yield sequencing techniques like whole-exome sequencing. Therefore, ordering diagnostic gene-sequencing panels on muscle diseases is not indicated in the setting of a clinical suspicion of FSHD.

CURRENT MANAGEMENT

Currently, no cure or pharmacologic treatments are available for FSHD; management focuses on supportive measures and surveillance of extramuscular complications. Patients may find orthotic devices like corsets for back support and leg bracing or orthoses for foot drop helpful.[30]

An evidence-based guideline developed by the American Academy of Neurology (AAN) and the American Association of Neuromuscular and

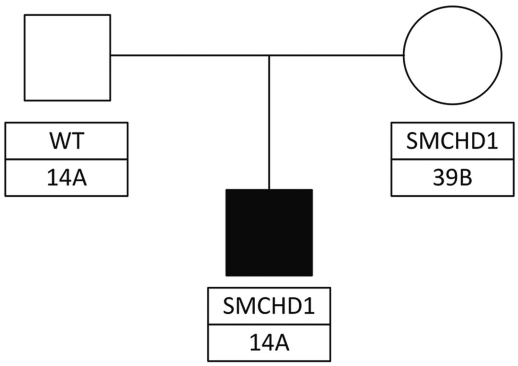

FIGURE 9-5

Pedigree of the patient in CASE 9-2. The top boxes represent the *SMCHD1* mutation status [*SMCHD1* variant present or wildtype (WT)]. The lower boxes show the number of D4Z4 repeat units and the 4q haplotype (A or B). The patient (*black square*) inherited both a pathogenic variant of the *SMCHD1* gene from his mother and a disease-permissive 4qA haplotype from his father. The presence of an *SMCHD1* pathogenic variant alone does not cause symptoms of facioscapulohumeral muscular dystrophy.

COMMENT

FSHD2 has an identical clinical phenotype to FSHD1 but has a digenic pattern of inheritance. Digenic inheritance is a nonmendelian form of inheritance in which pathogenic variants in two distinct genes are necessary to cause disease, and a pathogenic variant in either of the two genes alone is insufficient to lead to pathology. Consequently, in FSHD2 cases, family history is often negative. In FSHD2, D4Z4 repeat array sizes of 8 to 30 repeat units are found, which is longer than what would generally be seen in FSHD1, but shorter than in the general population. To cause disease, both a D4Z4 repeat array of 8 to 30 units on a 4qA haplotype and a pathogenic variant in a chromatin modifier gene (most often *SMCHD1*) are required.

Electrodiagnostic Medicine is available on the management of FSHD.[29] Recommendations include, among others, obtaining baseline pulmonary function tests on all patients with FSHD and monitoring respiratory function regularly in patients with severe proximal weakness, kyphoscoliosis, wheelchair dependence, or comorbid conditions that may affect ventilation. Routine cardiac screening is not indicated. Patients with a symptom onset in infancy, very short D4Z4 repeat array sizes (1 to 3 units), or both should be screened for hearing loss and retinal vascular disease.

Clinical trials have shown beneficial effects of aerobic exercise on physical fitness and chronic fatigue.[31-33] Physical activity may even slow disease progression in the leg muscles.[34] Therefore, FSHD patients should be encouraged to engage in low-intensity aerobic exercise, which can be any activity of their choosing including, but not limited to, biking, walking, or swimming. Moderate-intensity strength training appears to do no harm, but there is insufficient evidence to conclude that it offers benefit.[35]

Chronic musculoskeletal pain is a frequent complaint and inquiring about pain is indicated on every clinic visit. Patients can be referred to a physical therapist or be treated pharmacologically with nonsteroidal anti-inflammatory drugs for acute pain, or select antidepressants or antiseizure medications for chronic pain.[29]

A limited group of patients may benefit from scapular fixation, a surgical procedure to attach the scapula to the chest wall. These include patients with preserved deltoid strength, in whom manual scapular fixation at bedside examination results in a significant increase in shoulder range of motion. As the procedure is extensive and bears a risk of complications such as hemothorax or pneumothorax, nonunion, and decreased lung capacity, it should be offered cautiously to selected patients.

TARGETED THERAPY DEVELOPMENT

In the past, various clinical trials have been conducted on nontargeted pharmacologic interventions in FSHD, which all showed disappointing results. Clinical trials on albuterol, corticosteroids, creatine, diltiazem, and myostatin inhibitors failed to show clinical benefit.[36-43]

The advanced understanding that the misexpression of DUX4 is the mechanism underlying FSHD has led to opportunities for the development of targeted therapies that treat FSHD at its root cause. Various strategies are being explored to tackle either DUX4 expression or its downstream effects.

One compound that is currently undergoing clinical testing is an oral, selective, small-molecule inhibitor of p38α/β mitogen-activated protein kinase called losmapimod. Using pharmacologic screens of chemical libraries, β2-adrenergic agonists were identified as inhibitors of DUX4 expression, as shown by reduction of DUX4 target gene levels in FSHD myotubes.[44] During the search for signaling involved in β2-agonist repression of DUX4, it appeared that p38 mitogen-activated protein kinases are activated by β2-adrenergic signaling. The inhibition of DUX4 expression by p38 inhibitors seems to be independent of β2 agonists. Various preclinical studies confirmed that losmapimod reduces DUX4 expression significantly.[45] A multicenter phase 2 randomized, double-blind, placebo-controlled clinical trial on losmapimod (ReDUX4) has recently been completed.[46] Eighty patients with FSHD1 were randomized to receive either losmapimod (15 mg oral 2 times a day) or placebo for 48 weeks. The

primary endpoint, a change in *DUX4*-driven gene expression in muscle biopsies, was not met. However, differences favoring the losmapimod-treated patients were found on muscle MRI (slower progression of muscle fat infiltration) and multiple clinical outcome measures (improvement on reachable workspace, the Patients' Global Impression of Change scale, and fixed dynamometry of various muscles). Additionally, losmapimod appears to be safe and well tolerated. Following these promising outcomes, further studies on losmapimod will be pursued.

As mentioned previously, β2-adrenergic agonists have recently been demonstrated to inhibit DUX4 expression.[44] However, clinical trials on the β2-agonist albuterol only showed a small benefit on some of the clinical outcome measures. This is probably because the dose of albuterol needed to suppress DUX4 expression interferes with muscle regeneration.[36-38] Clenbuterol, a more potent β2 agonist, could potentially be of interest as an alternative without this adverse effect on myogenesis.[44]

Another way of modulating DUX4 expression is to target the DUX4 mRNA.

One approach is the use of antisense oligonucleotides that bind to complementary mRNA sequences, thereby either masking the sequence from the translation apparatus or initiating mRNA degradation.[47-51] Antisense oligonucleotides have been shown to effectively reduce DUX4 expression in both immortalized myocytes and a mouse xenograft model.[47,49,50,52] However, an important challenge that needs to be overcome for antisense oligonucleotides to be considered a viable therapeutic approach is their poor cell-penetrating ability, hindering sufficient delivery to the muscle tissue in vivo.[51]

Another approach to interfere directly with the DUX4 mRNA is vector-based RNA interference. RNA interference is a process of small noncoding RNAs (microRNAs) silencing the expression of other genes. MicroRNAs can be designed artificially to specifically target the DUX4 mRNA and then packaged within adeno-associated viral vectors for delivery into the muscle tissue.[53] These vector-based RNA interference strategies have the advantage of relatively easy delivery into the muscle tissue through the use of adeno-associated viral vector serotypes with high tropism for muscle. Proof-of-concept mouse studies on an adeno-associated viral–mediated microRNA against DUX4 showed promising results with reduced levels of DUX4 expression and an increase in grip strength after treatment.[54] A subsequent toxicology study in mice for two microRNAs targeting DUX4 demonstrated dose-dependent muscle toxicity in one but not the other, emphasizing the need for rigorous investigation of toxicity and off-target effects.[53]

CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) is being explored as a gene therapy approach for FSHD. This gene-editing technology allows for alteration of DNA sequences and modified gene function. It has been applied in FSHD2 myoblast cultures to correct deep intronic mutations in the *SMCHD1* chromatin modifier gene, resulting in an increase in SMCHD1 protein levels (but no restoration of D4Z4 methylation) and a reduction in DUX4 and DUX4 target gene expression.[55] In FSHD1, CRISPR technology with catalytically inactive or "dead" Cas9 was used to successfully silence the DUX4 promotor and exon 1 in myocytes.[56] More recent CRISPR/Cas9 approaches are focusing on targeting the 4qA polyadenylation signal to prevent stabilization of the DUX4 transcript.[57,58] Again, delivery of CRISPR/Cas9 to the muscle tissue remains a challenge. Additionally,

KEY POINTS

- **As FSHD1 is caused by a repeat contraction, it is not detected by high-yield sequencing techniques like gene-sequencing panels or whole-exome sequencing.**
- **Disease management of FSHD is currently supportive and outlined in evidence-based guidelines published by The American Academy of Neurology (AAN).**
- **Approaches for targeted therapies to reduce DUX4 expression that are currently being explored include small molecules, antisense oligonucleotides, vector-based RNA interference, and gene therapy.**

with the use of viral vectors, the issues of immunogenicity, safety, costs, and production capacity need to be resolved. However, the field of gene therapy is progressing quickly, and once these challenges can be overcome it may provide a promising targeted therapy for FSHD.

CLINICAL TRIAL PREPAREDNESS

In anticipation of upcoming clinical trials of newly developed therapies, several challenges need to be addressed.[4,59] Due to the generally slow yet highly variable rate of disease progression, either high numbers of participants or a long follow-up period will likely be required to show clinical benefit in drug trials. Therefore, it is of critical importance to develop biomarkers and outcome measures that are capable of capturing changes over the course of 1 year, a reasonable time frame for a clinical trial.

For early-phase trials, DUX4 target gene expression can be measured in cell cultures, animal models, and muscle biopsies, and is proposed as a biomarker for DUX4 activity.[60-63] DUX4 itself is not suited as a biomarker, as its expression in skeletal muscle is low and sporadic.[64,65] Multiple studies have shown that when DUX4 is knocked down, its target genes' expression decreases significantly.

Muscle MRI and ultrasound can be valuable as imaging biomarkers for clinical trials to assess the degree of fatty infiltration in the muscles.[17,66,67] Specific MRI sequences (short tau inversion recovery [STIR], T2 sequence with nulling of the fat signal) can be used to identify muscle edema, which is indicative of inflammation and can be found in approximately 5% of all muscles in patients with FSHD.[68] Inflammation in muscles in FSHD is a sign of active disease, and as such these muscles have a higher risk of rapid deterioration.[69,70] Therefore, inflamed muscles may be an attractive target for the testing of therapies and the presence of STIR-positive muscles could be used as an inclusion criterion for clinical trials.

Although it has been shown that both quantitative muscle MRI and ultrasound are able to detect changes in the degree of fatty infiltration of the muscle tissue over time, the clinical meaningfulness of these changes still needs to be established.[67,71,72]

Electrical impedance myography could serve as another noninvasive biomarker. It uses high-frequency, low-intensity electrical currents to assess changes in muscle composition. Although electrical impedance myography showed good reliability and correlation to clinical measures, its sensitivity to detect changes in FSHD is still uncertain.[73]

For later-phase trials, clinical outcome measures are required to demonstrate the effectiveness and clinical benefit of a therapy. Two promising functional outcome measures are the FSHD composite outcome measure (FSHD-COM) and reachable workspace.[74-76] The FSHD-COM is an 18-item physician-reported instrument that tests function throughout the body. The included functional motor tasks represent areas that were deemed important by patients with FSHD and include function of the leg, shoulder and arm, trunk, and hand, and balance. The reachable workspace uses a three-dimensional vision-based sensor system to assess the range of motion of the arms as a measure of gross shoulder function.

Two patient-reported outcome measures are the FSHD Rasch built overall disability scale (FSHD-RODS) and the FSHD health index (FSHD-HI). The FSHD-RODS is a 32-item scale that measures the level of daily activities and

social participation.[77] It has the advantage of being an interval scale, indicating that the differences between points on the scale are measurable and equal, therefore enabling parametric testing and comparison of changes throughout the scale. The FSHD-HI measures total FSHD health-related quality of life and 14 subdomains in areas that were identified as important by patients with FSHD.[78]

These outcome measures are all currently being evaluated in longitudinal studies to determine their sensitivity to change and minimal clinically important differences.[76]

KEY POINT

● **Due to the generally slow yet highly variable rate of disease progression in FSHD, the development of sensitive biomarkers and clinical outcome measures is of great importance to prepare for upcoming clinical trials.**

CONCLUSION

FSHD is one of the most prevalent muscular dystrophies. It has a distinct phenotype, but is variable in severity. Advances in the understanding of the genetic and epigenetic mechanisms underlying FSHD are paving the way for the development of targeted therapies, including small molecules, antisense oligonucleotides, vector-based RNA interference, and gene therapy approaches. Although the current management of FSHD is supportive, these are promising times for the FSHD community with various potential disease-modifying therapies on the horizon.

USEFUL WEBSITES

FSHD SOCIETY
The website from the USA FSHD patient organization includes information on FSHD for physicians, but especially for patients, including videos, articles, and resource lists.
fshdsociety.org

MUSCULAR DYSTROPHY ASSOCIATION
An informative website that provides information to patients, caregivers, and researchers on patient care, family support, and scientific advances in neuromuscular disease.
mda.org

FRIENDS OF FSHD RESEARCH
A research-focused website that includes information and scientific news about FSHD.
fshfriends.org

FSHD GLOBAL
The website from the Australian FSHD organization that provides, among other resources, an "FSHD Medical Education Portal" for people living with FSHD.
fshdglobal.org

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