

# Advancements in Diagnostics and Therapeutics in Facioscapulohumeral Muscular Dystrophy- a Systematic Review

Aleti Keerthana Reddy<sup>1</sup>, Preeti N<sup>2</sup>, Roshni Subramaniyan<sup>2</sup>, Harsha Vardhanan T<sup>2</sup>, Sahasya Adalarasan<sup>2\*</sup>, Kanika Balasubramani<sup>2</sup>

**Background:** Facioscapulohumeral muscular dystrophy (FSHD) is a genetic, typically autosomal dominant disorder causing progressive muscle weakness and atrophy, primarily affecting the face (facio-), shoulder blades (scapulo-), and upper arms (humeral). Recent advancements in FSHD have improved diagnostic and therapeutic strategies.

**Objective:** To provide a comprehensive review of current advancements in diagnostics and treatment in FSHD.

**Materials and Methods:** This review synthesizes findings from recent literature on advancements in FSHD, including molecular diagnostics, imaging techniques, application of artificial intelligence (AI), treatment strategies such as targeting DUX4 (Double Homeobox 4), role of si/miRNAs, gene editing, etc. A systematic approach was adopted to identify and analyze relevant studies.

**Results:** Key advancements under diagnostics include molecular diagnostics, flow cytometry-based diagnostics, Imaging-based diagnostics, artificial intelligence-based diagnostics, and various Biomarkers.

Key advancements under treatment include DUX-4 targeted therapies, muscle-enhancing and supportive therapies, emerging small-molecule and anti-inflammatory approaches, observational studies, and disease monitoring.

**Conclusion:** Recent innovations in FSHD have significantly expanded diagnostic and therapeutic possibilities. Emerging technologies, including AI and regenerative approaches, have immense potential for future advancements. Continued research and clinical integration of these innovations are critical for enhancing periodontal care and improving patient outcomes.

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## Introduction

Facioscapulohumeral Muscular Dystrophy was the first disorder identified to be linked with abnormal expression of the DUX4 gene. It is also the third most prevalent muscular dystrophy worldwide, with an estimated prevalence of 1 in 8,000-20,000 individuals depending on ancestry. Clinical manifestations usually appear before 20 years of age and are characterized by progressive skeletal muscle weakness, predominantly affecting the facial, shoulder girdle, upper trunk, and lower limb muscles. Despite the absence of a definitive cure, affected individuals generally have a normal life expectancy.[1]

FSHD develops exclusively in individuals carrying the D4Z4 4qA allele, which contains a non-canonical

polyadenylation signal within exon 3 that stabilizes DUX4 mRNA transcripts. This stabilization results in toxic accumulation of DUX4 protein in skeletal muscle cells. Two major genetic subtypes of FSHD have been described: FSHD1 and FSHD2. Approximately 95% of cases are classified as FSHD1, which arises due to contraction of the D4Z4 repeat array to fewer than 10 repeat units. This contraction disrupts chromatin organization and reduces repressive H3K9me3 methylation, thereby enabling aberrant activation of DUX4 in skeletal myocytes. In contrast, FSHD2 is caused by haploinsufficiency or loss-of-function mutations in genes responsible for D4Z4 methylation, leading to generalized hypomethylation across the D4Z4 array rather than restriction to the distal repeat. However, FSHD2 does not necessarily produce greater DUX4 expression or a more severe clinical

<sup>1</sup>Department of Genetics, Osmania University, India

<sup>2</sup>Medical Student, Institute of Internal Medicine, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, India

**Correspondence to:** Sahasya Adalarasan, Medical Student, Institute of Internal Medicine, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, India. E-mail: [surgeonsahasya@gmail.com](mailto:surgeonsahasya@gmail.com)

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phenotype compared with FSHD1. When combined with D4Z4 contraction, these mutations may further aggravate disease severity.[1]

Current evidence indicates that the overall cancer risk in patients with FSHD is not significantly higher than that of the general population. Nevertheless, some recent studies suggest a possible increase in the incidence of gastrointestinal malignancies among FSHD patients above 40 years of age.[1]

Identification of DUX4 rearrangements, particularly in DUX4-rearranged B-cell acute lymphoblastic leukemia (DUX4-r B-ALL), remains technically difficult. Standard cytogenetic approaches such as G-banded karyotyping, fluorescence in situ hybridization (FISH), and SNP array analysis often fail to detect these rearrangements because they are typically small, located near telomeric regions, and involve highly repetitive D4Z4 sequences that can cause cross-hybridization and false-negative findings.[1]

FSHD therapies are now focused on targeting DUX4, the pathogenic driver.[2,3] Preclinical advances (antisense oligonucleotides, siRNA/miRNA, gene editing) have shown dramatic DUX4 knockdown and improved muscle cell health[3,4]. A few drugs have reached patients: the p38 inhibitor losmapimod slowed fat infiltration on MRI and showed hints of functional benefit, but failed its primary molecular endpoint.[4,5] Drugs that merely enlarged muscle (like myostatin inhibitors) also failed to improve strength.[5,6] Repurposed agents (albuterol, diltiazem, vitamins) yielded at best modest or equivocal results.[3,5,7] The central lesson is that suppressing DUX4 or its toxicity may modify disease, whereas older anabolic or antioxidant strategies have weak evidence.

## Materials and Methods

### Study design

This review was conducted in accordance with the PRISMA guidelines to ensure transparent and reproducible reporting of methods and findings.

### Inclusion criteria

- Studies published in peer-reviewed journals between 2020 and 2026.
- Articles focusing on advancements in FSHD
- Publications in English.
- Original research, review articles, and meta-analyses.

### Exclusion criteria

- Studies unrelated to FSHD.
- Abstracts, editorials, or non-peer-reviewed content.

### Information sources

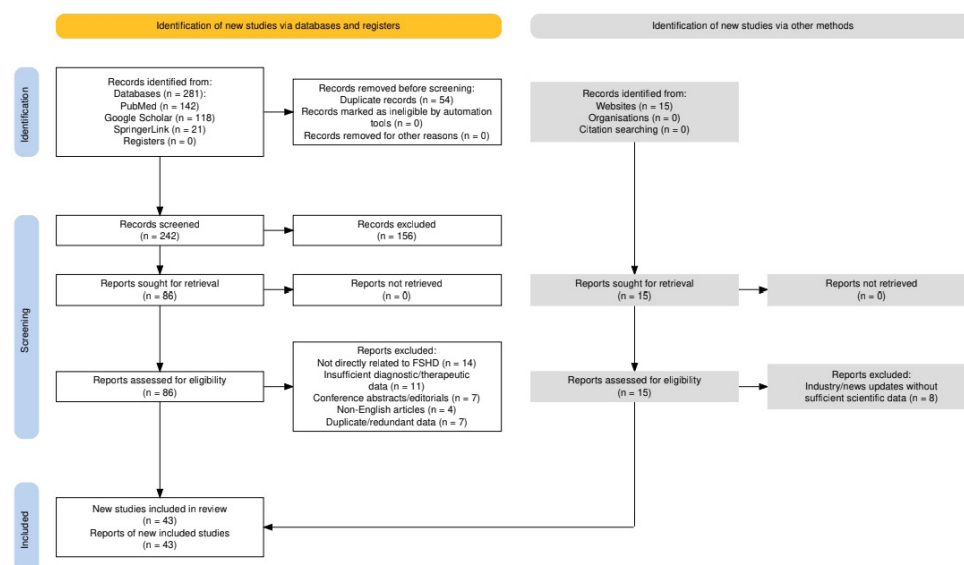
A systematic search was conducted in electronic databases, including PubMed and Google Scholar. Additional articles were identified through manual searches of the references cited in the included studies.

### Search strategy

The search strategy was developed using Boolean operators and Medical Subject Headings (MeSH) terms related to FSHD.

### Study selection

Titles and abstracts were independently evaluated by two reviewers according to the predefined inclusion criteria. Full-text versions of studies considered potentially eligible were then obtained and reviewed in detail. Any



Flow Chart 1: PRISMA Flow Chart [8]

disagreements between the reviewers were resolved through discussion, and when necessary, by consulting a third reviewer.

### *Data extraction*

Data were extracted independently by two reviewers using a standardized data extraction form. Key data items included:

- Author(s) and publication year.
- Study type and sample size.
- Key advancements in FSHD discussed.
- Outcomes and conclusions.

### *Data synthesis*

The extracted data were synthesized qualitatively, with a focus on advancements in molecular diagnostics, imaging techniques, artificial intelligence, etc. The findings were organized into thematic categories for discussion.

## **Results**

This systematic review and meta-analysis provides a comprehensive evaluation of the novel and recent innovations and advancements in FSHD. Our findings emphasize the significant progress made in understanding and diagnosing and treating FSHD, also highlighting both the potential and challenges of these innovations in clinical practice.

## **Diagnostics**

### *RNA sequencing tools*

RNA sequencing has become the most successful and widely used method for detecting DUX4 rearrangements. Many studies use fusion-detection software (such as STAR-Fusion and FusionCatcher) to identify fusion breakpoints from RNA-sequenced data. Differential gene expression analysis using tools (like DESeq2, along with clustering methods such as hierarchical clustering, PAM, and t-SNE) can identify the unique transcriptional profile of DUX4-r leukemia. Machine-learning approaches (such as ALLCatchR and ALLSorts) further improve subtype classification. These RNA-based methods consistently detect DUX4-r in approximately 5–10% of B-ALL cases.[1] Although DUX4-rearranged B-ALL is a distinct hematological malignancy rather than a manifestation of FSHD, studies of DUX4 detection technologies provide important methodological insights into detecting complex DUX4 rearrangements. These techniques are discussed here because they may inform future molecular diagnostic approaches applicable to DUX4-related disorders, including FSHD.

### *Short read whole genome sequencing*

Short-read whole genome sequencing has also been used successfully. The GRIDSS structural variant caller reconstructs breakpoint regions using contig assembly, helping overcome mapping difficulties caused by repetitive regions. Pelops was another tool specifically developed for DUX4-r detection. It identifies read pairs spanning DUX4 fusion junctions and normalizes them using a metric called SRPB (Spanning Read Pairs per Billion total reads) to account for sequencing depth differences. Pelops successfully identified all validated DUX4-r cases in pediatric B-ALL cohorts.[1]

### *Long read sequencing*

Long-read sequencing technologies provide even more precise characterization of DUX4 fusion events. 10x Genomics Chromium, PacBio SMRT sequencing, and Oxford Nanopore Technology (ONT) can map large fusion-containing DNA fragments spanning repetitive regions such as the Immunoglobulin heavy chain locus (IGH). Studies using PacBio successfully confirmed IGH::DUX4 rearrangements in the NALM6 cell line, while ONT adaptive sampling identified DUX4-r cases and associated ERG deletions in B-ALL patients. These approaches, however, are expensive. ONT sequencing initially had high error rates, but newer basecalling algorithms have greatly improved accuracy.[1]

### *Indirect methods*

Indirect methods are also used to identify DUX4-r cases. ERG (Erythroblastosis Transformation-Specific Regulated Gene 1) deletion (ERGdel) is commonly used as a surrogate marker because it can be detected by MLPA, SNP arrays, and PCR. However, ERGdel is absent in 40–60% of DUX4-r cases and can occasionally occur without DUX4 rearrangements, limiting its reliability. ERGalt expression has also been proposed as a marker, but its levels vary widely. [1]

### *1.5.CD371 Flow cytometry*

Detection of CD371 expression by flow cytometry is also a very reliable method. Most DUX4-r B-ALL cases express CD371, giving this method very high sensitivity and specificity. Combined CD371 and CD2 expression appears almost exclusive to DUX4-r leukemia, although CD2 is expressed only in about 50–75% of cases, making CD371 alone more sensitive. Because flow cytometry is inexpensive, rapid, and widely accessible, CD371 detection is considered highly useful in clinical settings with the lack of sequencing technologies.[1]

## 6. Ferroptosis in FSHD

Recent studies have also identified evidence of Ferroptosis in FSHD in muscle tissue. Biomarkers of ferroptosis are elevated in FSHD biopsies and correlate with inflammation and fatty infiltration. Increased labile iron and lipid peroxidation worsen plasma membrane repair in FSHD myoblasts, whereas treatment with the iron chelator 2,2'-Bipyridyl and the ferroptosis inhibitor ferrostatin-1 improves membrane repair. These findings suggest that ferroptosis contributes to FSHD pathology and represents a future therapeutic target.[9]

## Diagnosis with MRI

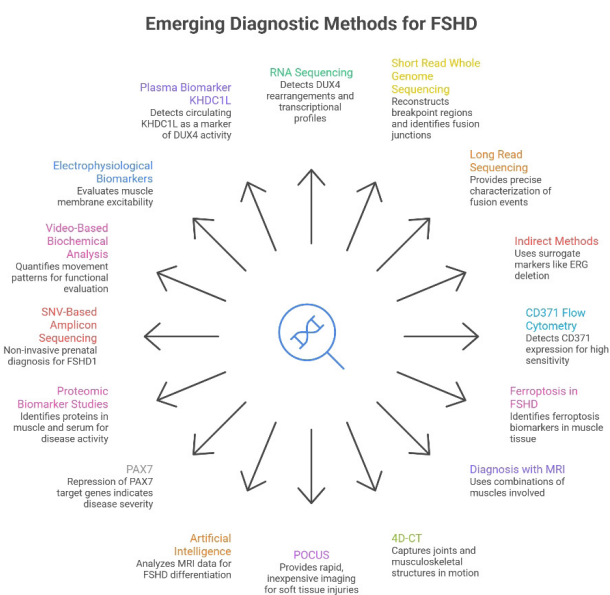
Researchers retrospectively analyzed MRI scans from 295 patients, including 187 patients with FSHD and 108 patients with other muscle diseases. Both upper-limb and lower-limb muscle MRIs were evaluated. The researchers examined 15 different radiological features and used a random forest machine-learning algorithm to determine which MRI findings were useful for distinguishing FSHD from other myopathies. It was found that the most useful MRI pattern for diagnosing FSHD was the combination of trapezius muscle involvement together with bilateral sparing of the subscapularis muscles, having very high diagnostic accuracy, with a sensitivity of 90% and specificity of 88%. This pattern was able to correctly identify 91% of patients who had atypical forms of FSHD. An even more specific pattern was identified when trapezius involvement was combined with bilateral sparing of both the subscapularis and iliopsoas muscles, along with asymmetric involvement of upper- and lower-limb muscles. This pattern was considered essentially pathognomonic for FSHD because it achieved a specificity of 99%, i.e., it was almost never seen in non-FSHD muscle disorders.[10]

## Dimensional CT (4D-CT)

It is also known as dynamic CT. Unlike static imaging, 4D-CT captures joints and musculoskeletal structures in motion and allows real-time analysis of joint kinematics. Although mainly discussed for orthopaedic applications, this concept may become relevant in FSHD because patients develop abnormal biomechanics, compensatory movement patterns, scapular instability, and gait abnormalities as muscles progressively weaken.[11] At present, evidence supporting 4D-CT in FSHD is theoretical and extrapolated from musculoskeletal imaging literature. No clinical studies have validated its routine diagnostic or monitoring role in FSHD.

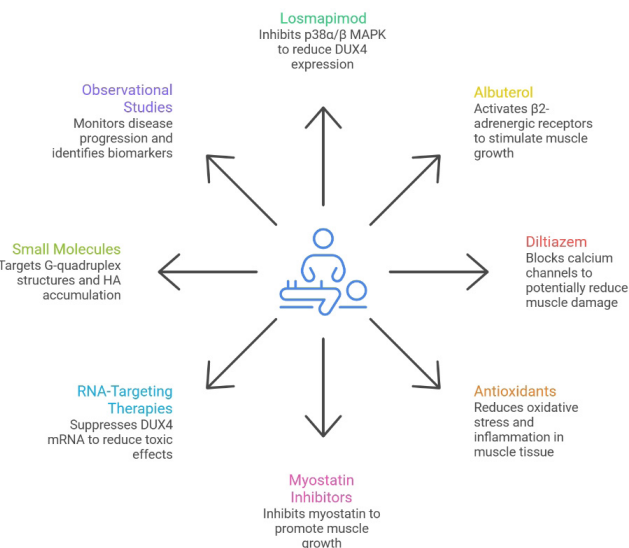
## POCUS

Point-of-Care Ultrasound (POCUS) provides rapid, inexpensive, real-time imaging without radiation



**Figure 1:** Mind map of advancements in diagnostics in FSHD

## FSHD Emerging Treatment Strategies



**Figure 2:** Mind map of advancements in treatment of FSHD

exposure. It is useful for evaluating soft tissue injuries and performing ultrasoundguided interventions such as corticosteroid injections. Standardized approaches like the ABSIS method have been developed for shoulder ultrasound examination. POCUS has shown high diagnostic accuracy for rotator cuff injuries and supraspinatus tendon tears, making it a valuable adjunct to clinical examination. Thus, it could potentially become useful for bedside assessment of muscle wasting, fatty replacement, or asymmetric muscle involvement,

especially in settings where MRI access is limited.[11] Similarly, the application of POCUS in FSHD remains speculative. Although ultrasound has proven utility in musculoskeletal disorders, dedicated validation studies in FSHD populations are currently lacking.

### *Artificial Intelligence*

Artificial intelligence (AI) and machine learning (ML) are becoming major components of musculoskeletal diagnostics. Recent advances in artificial intelligence have demonstrated the potential of deep learning (DL)-based analysis of whole-body muscle MRI for differentiating FSHD from inflammatory myositis without requiring manual grading of muscle signal abnormalities. In a retrospective study involving 19 genetically confirmed FSHD1 patients and 21 myositis patients, a supervised convolutional neural network was trained using T1-weighted and STIR whole-body MRI images from the thigh, calf, and shoulder regions.

Diagnostic performance improved with the inclusion of multiple anatomical regions, highlighting the importance of whole-body pattern recognition in FSHD1. The DL model achieved correct classification rates of 69% using thigh images alone, 75% using thigh and calf images, and 77% using combined thigh, calf, and shoulder images, with the latter configuration demonstrating a mean AUC of 0.86 (95% CI: 0.72–0.99). Importantly, the DL system achieved diagnostic performance comparable to 2 expert musculoskeletal radiologists, whose correct classification rates were 95% and 87.5%, with no statistically significant difference between the DL model and radiologists ( $P = 0.41$  and  $P > 0.99$ ). Moreover, among seven MRI examinations misclassified by radiologists, the DL tool correctly classified six cases, suggesting that AI may identify subtle disease-specific imaging patterns overlooked during visual interpretation. Unlike earlier machine learning approaches requiring manual muscle-by-muscle grading, this DL method directly analyzed raw MRI data without muscle segmentation or prior visual scoring, thereby reducing observer variability and postprocessing time. These findings support the feasibility of AI-assisted MRI interpretation as a promising adjunctive diagnostic tool for distinguishing FSHD1 from inflammatory myopathies, particularly in diagnostically challenging cases.[12]

### *PAX7*

PAX7 (Paired Box 7) is important for satellite cells and muscle regeneration, so repression of its target genes likely reflects impaired muscle repair mechanisms in

FSHD. A major finding was that repression of PAX7 target genes was a much better indicator of disease state and severity than DUX4 target gene expression. Studies showed that PAX7 target gene repression could clearly distinguish healthy muscle, inflamed FSHD muscle, and non-inflamed FSHD muscle, even after adjusting for age and sex. More importantly, the amount of PAX7 repression in non-inflamed muscle correlated with clinical severity scores. This is significant because non-inflamed muscle may represent earlier or more stable disease tissue, meaning the biomarker can reflect underlying disease burden rather than just acute inflammatory activity. It was also discovered that PAX7-related abnormalities in muscle correlated with similar changes detectable in blood cells. Based on this, the researchers developed a “FSHD muscle blood biomarker” composed of 143 selected PAX7 target genes measurable from blood samples. This biomarker successfully correlated with disease severity in both muscle and blood.[13]

### *12. Proteomic Biomarker Studies*

An MRI-targeted proteomic biomarker study used shotgun liquid chromatography-mass spectrometry (LC-MS) on muscle interstitial fluid obtained by microdialysis from 20 muscles of 10 genetically confirmed FSHD patients, differentiated into STIR+ (active/inflamed) and STIR- muscles, along with serum samples and controls. A total of 1156 proteins were identified by data-independent acquisition (DIA), including 130 proteins uniquely present in STIR+ muscles, while a second data-dependent acquisition (DDA) platform confirmed broad reproducibility with 67% overlap between methods. Proteomic profiles successfully differentiated FSHD muscles from controls and demonstrated significant inflammatory activation in STIR+ muscles. The proteins S100-A8, S100-A9, Dermcidin, Insulin-like growth factor binding protein-4, and Prostaglandin-H2 D-isomerase were significantly upregulated in STIR+ muscles, whereas structural muscle proteins and plasminogen-pathway proteins were downregulated, suggesting impaired muscle regeneration and increased fibrosis during early active disease. Complement and innate immune pathways were also activated, while myogenic regulators such as MYOD1, MEF2C, and the MRTF/SRF axis showed inhibition, further supporting the defective regenerative capacity. Importantly, S100-A8, S100-A9, and Dermcidin were consistently detected both in actively affected muscles and selectively in FSHD sera, making them promising circulating biomarkers of disease activity. Thus, it is demonstrated that MRI-guided microdialysis

**Table 1:** Summary of advancements in Diagnostics in FSHD

| Category                       | Details                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Molecular                      | <p>RNA sequencing: Widely used for detecting DUX4 rearrangements</p> <p>Diagnostics using fusion-detection tools such as STAR-Fusion and FusionCatcher, along with clustering and ML-based subtype classification. DUX4-r is identified in approximately 5-10% of B-ALL cases.[1]</p> <p>Short-read whole genome sequencing: GRIDSS and Pelops were used to reconstruct breakpoint regions and detect DUX4 fusion junctions despite repetitive genomic regions.[1]</p> <p>Long-read sequencing: Technologies such as PacBio SMRT sequencing, 10x Genomics Chromium, and ONT enabled precise mapping of IGH::DUX4 rearrangements and associated ERG deletions.[1]</p> <p>Indirect methods: ERG deletion and ERGalt expression were used as surrogate markers for DUX4-r detection through MLPA, SNP arrays, and PCR, although sensitivity was limited.[1]</p> <p>CD371 flow cytometry: CD371 expression showed high sensitivity and specificity for identifying DUX4-r B-ALL and provided a rapid, inexpensive alternative where sequencing facilities were unavailable.[1]</p> <p>SNV-based amplicon sequencing: Maternal cfDNA screening with multiplex PCR-based haplotype analysis achieved 100% concordance with invasive prenatal testing in high-risk FSHD1 pregnancies.[15]</p> |
| Imaging Techniques             | <p>Optical genomic mapping (OGM): OGM simultaneously detected D4Z4 repeat contractions and 4q haplotypes and was considered simpler and more convenient than Southern blotting.[15]</p> <p>MRI diagnosis: Characteristic MRI patterns, especially trapezius involvement with bilateral sparing of subscapularis muscles, demonstrated high diagnostic accuracy for FSHD (sensitivity 90%, specificity 88%).[10]</p> <p>4D-CT: Dynamic CT enabled real-time assessment of joint kinematics and may help evaluate abnormal biomechanics and scapular instability in FSHD patients.[11]</p> <p>POCUS: Point-of-care ultrasound provided rapid and inexpensive bedside assessment of muscle wasting, fatty replacement, and asymmetric muscle involvement.[11]</p> <p>Video-based biomechanical analysis: Smartphone-based OpenCap analysis identified disease-specific gait and movement abnormalities and outperformed traditional timed function tests in disease classification.[16]</p>                                                                                                                                                                                                                                                                                               |
| Artificial Intelligence (AI)   | <p>Deep learning-based whole-body MRI analysis differentiated FSHD from inflammatory myopathies with diagnostic performance comparable to expert musculoskeletal radiologists. AI-based analysis reduced observer variability and eliminated the need for manual muscle grading.[12]</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Biomarkers and Pathophysiology | <p>Ferroptosis biomarkers: Elevated ferroptosis markers correlated with inflammation and fatty infiltration, while ferroptosis inhibitors improved membrane repair in FSHD myoblasts.[9]</p> <p>PAX7 biomarker: Repression of PAX7 target genes strongly correlated with disease severity, leading to the development of a minimally invasive blood-based “FSHD muscle-blood biomarker.”[13]</p> <p>Proteomic biomarkers: MRI-guided LC-MS proteomics identified inflammatory and fibrosis-associated proteins such as S100-A8 and S100-A9 as potential circulating biomarkers of active disease.[14]</p> <p>Electrophysiological biomarkers: MVRC and frequency ramp recordings demonstrated altered muscle membrane excitability, suggesting early electrophysiological biomarkers for disease monitoring.[17]</p> <p>Plasma biomarker KHDC1L: Elevated plasma KHDC1L levels reflected systemic DUX4 activity and showed promise as a minimally invasive biomarker for monitoring disease progression and therapeutic response.[18]</p>                                                                                                                                                                                                                                              |

combined with high-resolution proteomics can identify molecular changes before overt fatty muscle replacement occurs, and therefore can be used for minimally invasive disease monitoring and biomarker discovery in FSHD clinical trials.[14]

### *SNV (single nucleotide variant)-based amplicon sequencing*

Another recent non-invasive prenatal diagnostic approach for FSHD1 used maternal cell-free DNA (cfDNA) screening combined with multiplex PCR-based haplotype analysis targeting 402 high-frequency SNV sites within 2 Mb (Megabase) upstream of the *DUX4* locus. In a prospective study of 13 high-risk FSHD1 families, all pregnancies  $\geq 7$  weeks of gestation underwent cfDNA analysis, with results validated using invasive prenatal diagnosis by Karyomap gene chips

and Bionano single-molecule optical genomic mapping (OGM). The method successfully identified 8 affected and 5 unaffected fetuses with 100% concordance with invasive testing, achieving 100% diagnostic accuracy. The average gestational age at testing was 9 weeks (range 7+0–11+2 weeks). Reports were generated within 7 days, offering substantially faster turnaround, lower cost, earlier detection, and reduced procedural risk compared with traditional invasive prenatal diagnostic methods. OGM is a new technology that serves to be a simpler and more convenient method than southern blotting and can detect repeat contraction and 4q haplotypes simultaneously. In India, OGM-based testing is currently being offered at CSIR-Centre for Cellular and Molecular Biology, Hyderabad, a public institution, and also by a few private diagnostic laboratories.[15]

### ***Video-Based Biochemical Analysis***

A recent digital diagnostic advancement in FSHD utilized smartphone video-based biomechanical analysis to quantify three-dimensional movement patterns using OpenCap, a free open-source motion analysis platform. In an observational study involving 129 participants, including 28 FSHD patients, biomechanical data from nine functional movements were captured in a median time of 16 minutes per participant, generating 34 interpretable movement features. The system demonstrated excellent reproducibility of results with standard timed function tests (TFTs), including the 10-meter walk, 10-meter run, timed up-and-go, and 5-times sit-to-stand tests, reproducing all four with very high correlation ( $r > 0.98$ ) and similar test-retest reliability. But more importantly, video-based biomechanical analysis significantly outperformed TFTs in disease classification ( $P = 0.021$ ) and identified disease-specific movement signatures, including distinct gait kinematic abnormalities not detectable through standard TFTs. These findings suggest that smartphone-based biomechanical assessment may provide a sensitive, rapid, and clinically scalable tool for functional evaluation, disease monitoring, and clinical trial outcome assessment in FSHD.[16]

### ***Electrophysiological Biomarkers***

A novel electrophysiological biomarker approach in FSHD evaluated muscle membrane excitability using muscle velocity recovery cycle (MVRC) and frequency ramp recordings in the tibialis anterior and trapezius muscles. Recordings from 20 FSHD patients were compared with healthy controls (74 tibialis anterior and 33 trapezius controls). FSHD muscles demonstrated significantly reduced early and late muscle supernormality and blunted frequency ramp latency changes, indicating altered muscle membrane electrical properties and probable resting membrane depolarization. Moderate correlation was observed between late supernormality changes in the trapezius muscle and quadriceps muscle strength measured by dynamometry. Although correlations with overall disease severity were limited, the findings suggest that abnormalities in muscle membrane excitability occur early in the disease course and may serve as potential electrophysiological biomarkers for disease monitoring and future clinical trials in FSHD.[17]

### ***Plasma Biomarker KHDC1L***

A recent biomarker-based advancement in FSHD diagnostics identified plasma KHDC1L as a potential minimally invasive circulating biomarker of DUX4

activity. Since direct measurement of DUX4 in muscle tissue is difficult and muscle biopsy is invasive, researchers used mass spectrometry on conditioned media from DUX4-inducible immortalized human myoblasts (MB135iDUX4) to identify the secreted DUX4-regulated proteins. KHDC1L emerged as the top biomarker, and western blot analysis confirmed its release from DUX4-expressing cells. Subsequent plasma profiling further demonstrated elevated KHDC1L levels in FSHD patients compared with healthy controls, thereby suggesting that circulating KHDC1L reflects systemic DUX4 activity across affected muscles. Thus, plasma KHDC1L is also a potential plasma biomarker for disease monitoring and for assessing therapeutic response in emerging FSHD treatments, offering a less invasive alternative to muscle biopsy-based biomarkers.[18]

## **Treatment**

### ***Losmapimod and p38 Inhibitors***

Losmapimod inhibits  $p38\alpha/\beta$  MAPK, an important regulator of DUX4 expression during muscle differentiation [4]. In preclinical cell and animal models, it significantly suppressed DUX4 expression without interfering with muscle formation. The Phase 2 ReDUX4 trial randomized 80 patients with FSHD1 to receive losmapimod or placebo at 15 mg twice daily [19]. Although the study did not show a significant overall reduction in DUX4-driven gene expression after 16 weeks, patients with high baseline DUX4 activity demonstrated an approximately 38-fold reduction in DUX4 target genes compared with a 5-fold reduction on placebo [19]. The drug also showed trends toward slower fat replacement on MRI and mild improvements in shoulder movement, strength, and patient-reported outcomes, although these findings did not reach statistical significance [19,20]. Losmapimod was generally well tolerated, with mostly mild adverse effects such as headache and gastrointestinal symptoms [20]. However, a later Phase 3 REACH trial involving 260 adults with FSHD1 or FSHD2 failed to meet both primary and secondary endpoints, leading Fulcrum Therapeutics to suspend further development of losmapimod in 2024 [21].

### ***$\beta_2$ -Adrenergic Agonists (Albuterol)***

Albuterol is a short-acting  $\beta_2$ -adrenergic receptor agonist primarily used to treat asthma, but it has also been investigated in FSHD because of its anabolic effects on skeletal muscle.[5] Activation of  $\beta_2$ -adrenergic receptors increases cAMP production and stimulates

pathways involved in muscle hypertrophy and protein synthesis, including PKA and PI3K/Akt/mTOR, while reducing protein degradation [22]. Experimental studies also suggest that  $\beta_2$  agonists such as albuterol and clenbuterol may decrease DUX4 mRNA synthesis.[3,22] Trials conducted by Kissel et al. demonstrated modest but statistically significant increases in lean body mass and improvement in elbow flexor strength compared with placebo, while overall improvements in muscle strength and functional outcomes were inconsistent [22]. Albuterol was generally well tolerated, with common adverse effects including tremors, nervousness, cramps, and headache.[3,22] Although the anabolic effects appear promising, current evidence does not clearly support a major functional benefit in FSHD, and more potent  $\beta_2$  agonists such as clenbuterol may require further evaluation.

#### *Calcium-Channel Blockers (Diltiazem)*

Diltiazem, a calcium channel blocker commonly used to treat hypertension, has also been explored as a potential therapy for FSHD.[5,23] Its use was based on the hypothesis that abnormal calcium signaling may contribute to muscle fiber damage in muscular dystrophies by impairing mitochondrial function and increasing inflammatory activity.[5] However, studies evaluating diltiazem in FSHD did not show significant improvements in muscle strength, functional performance, or muscle mass. The drug was generally well tolerated, with no major adverse effects reported. Overall, the available evidence suggests that diltiazem has limited clinical benefit in FSHD, although further research may still be needed to better understand its potential role.[5]

#### *Antioxidants and Supplements*

Oxidative stress is believed to contribute significantly to muscle damage in FSHD, with studies showing increased reactive oxygen species (ROS), mitochondrial dysfunction, inflammation, and impaired antioxidant balance in affected muscle tissue [4,24]. DUX4 expression has also been linked to disruption of glutathione-related antioxidant pathways, further increasing cellular susceptibility to oxidative injury [4]. Based on this mechanism, antioxidant therapies, including vitamin C, vitamin E, zinc gluconate, selenium, and compounds such as tempol, have been investigated as supportive treatments [5,7]. Experimental studies demonstrated reduced DUX4-induced toxicity and improved muscle cell morphology, while clinical studies reported modest improvements in quadriceps strength, endurance,

and oxidative stress markers in some patients [5,7]. However, benefits in functional outcomes, such as walking performance, remained inconsistent, and current evidence suggests that antioxidant therapy may provide limited supportive benefit rather than a definitive disease-modifying effect in FSHD [5,7,24].

#### *Myostatin Inhibitors (ACE-083, MYO-029)*

MYO-029 is a monoclonal antibody designed to inhibit myostatin, a natural negative regulator of muscle growth, making it a potential therapeutic target in FSHD and other muscle-wasting disorders [5]. Clinical studies showed that although MYO-029 increased muscle fiber diameter, it did not significantly improve muscle strength or functional outcomes [5,25]. Adverse effects were more common at higher doses and included hypersensitivity reactions such as rash and urticaria, as well as infections, arthralgia, and pain related to immune-mediated effects [5]. The study's limited duration and relatively small sample size may have reduced the ability to detect meaningful functional changes [25]. Similar findings were observed with ACE-083, another myostatin-pathway inhibitor, which demonstrated significant increases in total and contractile muscle volume in both the biceps brachii and tibialis anterior muscle groups, along with reduced fat fraction in some patients [25]. However, these structural improvements failed to yield consistent functional or patient-reported benefits, even after prolonged treatment up to 12 months [25]. The most common adverse events with ACE-083 were mild to moderate injection-site reactions [25]. These findings suggest that increasing muscle mass alone may not be sufficient to restore functional recovery in FSHD [5,25]. Another anti-myostatin strategy currently under investigation is RO7204239, a monoclonal antibody developed by Hoffmann-La Roche that blocks myostatin to promote muscle growth. A phase II clinical trial (MANOEUVRE) is currently evaluating its effects on quadriceps muscle volume in 48 ambulatory adults with FSHD1 or FSHD2 [26].

Although clinical efficacy data are still pending, RO7204239 reflects the continued interest in targeting myostatin pathways as a supportive therapeutic strategy in FSHD [26].

RNA-Targeting Therapies (Antisense, siRNA, miRNA, CRISPR)

#### *RNA-silencing therapies*

RNA-based therapies remain one of the most promising targeted treatments for FSHD because they directly

suppress DUX4, the primary pathogenic protein driving disease progression [2,6,7,27]. Several approaches, including siRNAs, miRNAs, antisense oligonucleotides (AOs), PMOs, morpholinos, and LNA gapmers, have been developed to reduce DUX4 mRNA and limit its downstream toxic effects [2,28]. miRNA-based therapies such as miDUX4 and endogenous miR-675 significantly reduced DUX4 expression, decreased cellular toxicity, and protected muscle tissue in animal models [4,6,7]. miR-675 was also identified as a natural suppressor of DUX4, as inhibition of endogenous miR-675 increased DUX4-related gene expression in FSHD muscle cells [6,29]. Viral vector delivery systems improved targeting efficiency, and newer AAV-based delivery approaches have further expanded this field [4,30]. However, immune reactions, toxicity, off-target effects, repeat dosing, and delivery challenges remain important limitations [4,30].

#### *Antisense oligonucleotides and gapmer therapy*

Antisense oligonucleotide therapies have also shown promising preclinical results [31,32]. These synthetic nucleic acids bind DUX4 mRNA and either block translation or promote degradation through RNase H-mediated cleavage [6,7]. Platforms such as siRNAs, PMOs, morpholinos, and LNA gapmers significantly reduced DUX4 expression in FSHD myoblasts and mouse models [28,31]. LNA gapmers achieved up to an 84% reduction in DUX4 mRNA and improved muscle fusion and structure in vitro [4,28]. In animal studies, antisense therapies reduced fibrosis, inflammatory changes, and DUX4 target gene expression, with partial improvements in muscle histology and functional performance [6,7]. Delivery remains one of the biggest barriers because nucleic acids do not readily cross skeletal muscle membranes [2,6,7]. To improve delivery, researchers are exploring lipid nanoparticles, dendrimers, antibody-mediated systems, transferrin receptor targeting, and AAV-based vectors [30,33].

#### *New targeted siRNA platforms*

Newer RNA interference therapies are now moving closer to clinical use. AOC 1020 is a transferrin receptor-targeted siRNA designed to enter muscle cells and degrade DUX4 mRNA [34]. Early results from the FORTITUDE trial showed that it was well tolerated and seemed to reduce DUX4-regulated gene expression while improving muscle strength [34]. ARO-DUX4 is another siRNA candidate, and in mouse models it reduced DUX4 mRNA, lowered DUX4 target gene expression, and improved weight, muscle atrophy, and muscle function

[35]. DYNE-302 uses a similar antibody-conjugated siRNA platform and showed prolonged suppression of DUX4 expression with improved muscle structure and function in preclinical models [36]. Kate Therapeutics is also developing an AAV-delivered siRNA platform called MyoAAV for skeletal muscle DUX4 silencing [37]. Another RNA-based candidate, MC-DX4, is being developed through a collaboration between miRecule and Sanofi and combines RNA-targeting technology with antibody-mediated delivery to improve muscle uptake [38]. Together, these studies show that RNA silencing is becoming more precise and more deliverable, but long-term safety, durability, immune effects, and cost still need to be solved [34-38].

#### *Indirect RNA-targeting and gene-regulatory approaches*

Some newer therapies aim to suppress DUX4 indirectly by targeting its regulators rather than DUX4 itself. Researchers identified SIX1, SIX2, and SIX4 as transcription factors that promote abnormal DUX4 expression in FSHD muscle cells, and siRNA-mediated knockdown of these factors significantly reduced DUX4 and DUX4 target gene expression in cellular models [39]. This is important because it provides another way to lower DUX4 activity without directly binding DUX4 mRNA. Epigenetic approaches are also being developed because FSHD is associated with hypomethylation of the D4Z4 region and abnormal chromatin relaxation. EPI-321 is a CRISPR-derived epigenetic therapy designed to restore D4Z4 methylation without cutting DNA [40]. In FSHD muscle cell models, EPI-321 suppressed DUX4 expression and reduced muscle cell death, and early clinical development is now being prepared [40]. These approaches are still at an early stage, but they strengthen the idea that correcting the upstream gene-regulatory defect may be more effective than only blocking the final transcript [39,40].

#### *CRISPR-based RNA silencing and muscle regeneration strategies*

CRISPR-based RNA-silencing strategies have also shown promise in FSHD [4,7]. CRISPR-Cas13 targets RNA rather than DNA, reducing the risk of permanent genomic damage [4]. Preclinical studies showed marked suppression of DUX4 expression and reduced cell death in vitro and in vivo [4]. Other CRISPR approaches targeting SMCHD1 mutations or the DUX4 polyadenylation signal also reduced DUX4 transcriptional activity [7]. At the same time, not all new therapies work by direct DUX4 suppression. SAT-3247, for example, targets the

AAK1 protein and aims to improve muscle regeneration independently of DUX4 signaling[41]. In mouse models of FSHD-associated muscle degeneration, it improved muscle strength [41]. Overall, these approaches show how FSHD research is expanding beyond simple gene silencing toward broader muscle-targeted and regeneration-based strategies, but most of these methods still need stronger validation of delivery efficiency, durability, and long-term safety [4,7,41].

### *Other Targeted Small Molecules*

Several targeted small-molecule approaches are under investigation in FSHD to suppress DUX4 activity or mitigate its downstream toxic effects. One important

strategy involves G-quadruplex (GQ) ligands. GQ structures were identified within the DUX4 enhancer, promoter, and transcript regions using predictive software and later confirmed experimentally by NMR and circular dichroism [4,32]. Berberine, a natural alkaloid and GQ ligand, showed strong binding to these regions and produced dose-dependent suppression of DUX4 mRNA in FSHD myoblasts [32]. It also reduced expression of downstream DUX4 target genes, including ZSCAN4, TRIM43, and MBD3L2, while improving myotube fusion and differentiation [32]. Additional studies with the GQ ligand PhenDC3 likewise suppressed DUX4 expression, supporting the idea that targeting GQ motifs may regulate DUX4 transcription and RNA stability. In

**Table 2:** Summary of advancements in Treatment of FSHD

| Category                                           | Details                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Losmapimod (p38 inhibitor)                         | ReDUX4 Phase 2b trial involving 80 FSHD1 patients demonstrated approximately 38-fold reduction in DUX4 target genes in patients with high baseline DUX4 expression, along with slower MRI fat infiltration and mild functional improvement, although the primary molecular endpoint was not met. The later REACH Phase 3 trial failed to achieve primary and secondary endpoints, leading to discontinuation of development in 2024.[19-21] |
| $\beta_2$ -Adrenergic Agonists (Albuterol)         | Studies by Kissel et al. and van der Kooi et al. showed modest increases in lean body mass and elbow flexor strength, but overall functional outcomes remained inconsistent. Adverse effects were generally mild, including tremors and nervousness.[22]                                                                                                                                                                                    |
| Myostatin Inhibitors (MYO-029, ACE-083, RO7204239) | MYO-029 increased muscle fiber diameter without significant improvement in strength or function. ACE-083 increased total and contractile muscle volume but failed to produce consistent functional benefit, resulting in discontinuation of development. RO7204239 is currently under evaluation in the MANOEUVRE Phase II trial.[25,26,27]                                                                                                 |
| miRNA and siRNA Therapies                          | Preclinical studies involving miDUX4, miR-675, AOC 1020, ARO-DUX4, and DYNE-302 demonstrated suppression of DUX4 expression, reduction in downstream target genes, and improvement in muscle pathology in animal models. Early FORTITUDE trial results with AOC 1020 also showed reduced DUX4-regulated gene expression and possible strength improvement.[7,34-41]                                                                         |
| Antisense Oligonucleotides and LNA Gapmers         | Multiple studies demonstrated up to 84% reduction in DUX4 mRNA expression with improvement in muscle fusion, histology, and fibrosis reduction in cellular and mouse models. Delivery to skeletal muscle remains a major limitation.[20,21,28]                                                                                                                                                                                              |
| CRISPR-Based Therapies                             | CRISPR-Cas13 and epigenetic CRISPR approaches such as EPI-321 significantly reduced DUX4 expression and muscle toxicity in preclinical studies without permanent DNA editing. These approaches remain experimental but highly targeted.[4,7,41]                                                                                                                                                                                             |
| G-Quadruplex Ligands (Berberine)                   | Berberine and related G-quadruplex ligands reduced DUX4 transcription and downstream target gene expression in preclinical studies, with associated reduction in fibrosis and improvement in muscle force in mouse models.[32]                                                                                                                                                                                                              |
| 4-Methylumbelliferone (4MU)                        | Preclinical studies showed that inhibition of hyaluronic acid synthesis reduced mitochondrial dysfunction, cellular abnormalities, and downstream DUX4-associated pathology despite minimal direct suppression of DUX4 itself.[33]                                                                                                                                                                                                          |
| Inflammation-Targeted Therapy (Satralizumab)       | Elevated IL-6 levels and chronic inflammatory activity identified in FSHD patients led to initiation of a clinical trial evaluating satralizumab as a potential anti-inflammatory therapy for slowing disease progression.[26]                                                                                                                                                                                                              |
| Observational and Natural History Studies          | Studies including ReSolve, ADVANCED, WANTED, PROGRESS-FSHD, and INSIGHT-FSHD2 are evaluating MRI biomarkers, wearable monitoring, smartphone-based assessments, pulmonary involvement, and functional outcome measures to improve future FSHD therapeutic trial                                                                                                                                                                             |

**Table 3:** Clinical trial status of selected therapeutic approaches in FSHD

| <i>Intervention</i> | <i>Stage</i>                  | <i>Evidence</i> | <i>Sample Size</i> | <i>Main Outcome</i>                    | <i>Clinical Status</i>   |
|---------------------|-------------------------------|-----------------|--------------------|----------------------------------------|--------------------------|
| Losmapimod          | Phase III                     | RCT             | 260                | Failed primary endpoint                | Development discontinued |
| ACE-083             | Phase II                      | RCT             | ~50                | ↑ muscle volume, no functional benefit | Discontinued             |
| MYO-029             | Phase I/II                    | Clinical trial  | Small              | No functional benefit                  | Not developed            |
| Antioxidants        | Small clinical studies        | Low-moderate    | Variable           | Modest biochemical benefit             | Supportive only          |
| AOC1020             | Early clinical                | Phase 1/2       | Small              | Preliminary DUX4 suppression           | Ongoing                  |
| DYNE302             | Preclinical                   | Animal          | -                  | Reduced DUX4                           | Experimental             |
| CRISPR              | Preclinical                   | Animal/cell     | -                  | DUX4 suppression                       | Experimental             |
| EPI-321             | Preclinical/early development | Cell models     | -                  | Epigenetic silencing                   | Experimental             |

mouse models overexpressing DUX4, berberine treatment improved muscle force and significantly reduced fibrosis, although effects on muscle regeneration were limited. The evidence remains preclinical [4,32].

Another downstream therapeutic strategy targets hyaluronic acid (HA) accumulation. DUX4 activity increases HA production, which contributes to cellular abnormalities and mitochondrial dysfunction in FSHD muscle cells [33]. The HA synthesis inhibitor

4-methylumbelliferone (4MU) reduced HA accumulation, improved cellular morphology, and decreased mitochondrial disturbances without directly lowering DUX4 protein levels [3,33]. These findings suggest that blocking downstream pathological pathways may still provide therapeutic benefit even if DUX4 expression is not fully suppressed. However, most evidence for this approach currently comes from cellular and preclinical studies [33].

Inflammatory pathways have also emerged as potential therapeutic targets in FSHD. An initial study involving blood samples from 100 adults with FSHD1 demonstrated chronic systemic inflammation, including elevated levels of pro-inflammatory cytokines such as interleukin-6 (IL-6), which may serve as a biomarker of disease activity [26]. Based on these findings, CHU de Nice launched a clinical trial in January 2024 to evaluate satralizumab, a monoclonal antibody targeting IL-6, with the aim of reducing inflammation and slowing disease progression in FSHD patients [26].

#### **Observational studies currently being conducted :**

Observational studies play an important role in facioscapulohumeral muscular dystrophy (FSHD) because the disease progresses slowly and shows significant clinical variability between patients. Several

ongoing studies in France are focused on improving disease monitoring, identifying biomarkers, and developing reliable outcome measures for future clinical trials. The international ReSolve study is evaluating tools such as the FSHD Composite Outcome Measure (FSHD COM) and electrical impedance myography to better assess disease progression. The French arm of the study, ReSolve\_France, is being conducted in centers including Nice, Lille, and Paris. Other important studies include the ADVANCED study for non-ambulatory patients, the WANTED study assessing motor function and wearable activity monitoring, and the PROGRESS-FSHD study, which uses a smartphone application for remote monitoring of fatigue, pain, sleep, and physical activity. Another major contribution from France is the national FSHD registry, the Observatoire National Français des patients atteints de DMFSH, established in 2013 with support from AFM-Téléthon. The registry collects genetic and clinical information from patients with FSHD1 and FSHD2 and helps researchers study disease natural history, genotype phenotype correlations, epidemiology, and recruitment for therapeutic trials. In addition, the INSIGHT FSHD2 study is investigating inflammatory biomarkers and clinical outcome measures using blood investigations, muscle MRI, pulmonary function testing, and functional assessments to better understand disease progression.

Outside France, countries such as the United Kingdom, the United States, and China have also established national FSHD registries and observational programs. These studies contribute important long-term clinical data and support international collaborative research. An American registry-based study evaluating pulmonary involvement in FSHD found that respiratory

insufficiency, although usually mild, can occur in a subset of patients, emphasizing the need for regular respiratory monitoring. Overall, observational studies and registries are essential for improving understanding of FSHD progression and for supporting the development and evaluation of newer targeted therapies.

## Limitation

A notable limitation of this review is that majority of the studies included were observational, with considerable heterogeneity in sample sizes, study designs, methodologies, etc.. In addition, many studies did not have extended follow-up periods, limiting the ability to determine the long-term efficacy and sustainability of emerging therapeutic approaches. Future investigations should prioritize large-scale randomized controlled trials to better evaluate the durability and clinical effectiveness of various diagnostic tools. Moreover, efforts toward standardizing regenerative therapy protocols and enhancing the availability of advanced imaging and artificial intelligence-based technologies will be extremely essential in facilitating their widespread integration into routine clinical practice.

## Conclusion

In essence, recent advances in FSHD diagnostics have significantly improved the ability to detect, characterize, and monitor the disease through a set of molecular, imaging, artificial intelligence-based approaches, etc. Emerging technologies such as RNA sequencing, longread sequencing, AI-assisted MRI analysis, various circulating biomarkers, and video-based biomechanical assessment provide highly sensitive, less invasive, and more clinically scalable methods for diagnosis and disease monitoring. They also contribute to a better understanding of FSHD pathophysiology, thereby supporting the development of targeted therapies and more personalized patient management strategies.

With regards to treatment, the evidence indicates that therapies directly targeting DUX4 or its pathways are most promising. Losmapimod demonstrated safety and a hint of effect in a subgroup, so further study (possibly in longer or enriched trials) is justified. RNA-based therapies have demonstrated encouraging preclinical efficacy and early clinical feasibility. However, their long-term safety, delivery efficiency, durability of response, and clinical effectiveness remain to be established in adequately powered human trials. Future trial designs should use modern outcome measures (MRI fat fraction, composite scales) and target patients with measurable

disease activity. By focusing on the DUX4 mechanism and improving trial efficiency, the field can best translate these experimental therapies into patient benefit.

## Discussion

While numerous advances have been reported in FSHD diagnostics and therapeutics, their levels of clinical maturity differ substantially. Molecular diagnostics such as genetic testing and MRI-based pattern recognition are increasingly incorporated into clinical practice, whereas AI-assisted imaging, circulating biomarkers, RNA-targeting therapies, and gene-editing approaches remain largely investigational. Therefore, the findings presented in this review should be interpreted according to the current stage of evidence rather than assuming equivalent clinical applicability.

Many studies discussing about the diverse range of advances in FSHD were included in this systematic review and meta-analysis. These were published between 2020 and 2026. These were categorized into major domains such as molecular diagnostics, flow cytometry based diagnostics, Imaging based diagnostics, Artificial Intelligence, and various Biomarkers under diagnostics. Key advancements such as DUX-4 targeted therapies, Muscle Enhancing therapies, Emerging small molecule and anti inflammatory approaches, Observational studies and Disease monitoring were discussed under treatment.

### *Geographic distribution*

Most studies originated from European nations such as the UK, France, and Italy. Studies from China and the US are also included.

### *Study types*

Includes studies such as clinical trials, observational studies, systematic reviews, and metaanalyses, with sample sizes ranging from small pilot studies to large-scale trials.

## Advancements in Diagnostics in FSHD

### *Molecular Diagnostics*

#### *RNA sequencing tools*

RNA sequencing is the most commonly used method for detecting DUX4 rearrangements. Fusion-detection tools such as STAR-Fusion and FusionCatcher identify fusion breakpoints, while clustering and machine-learning methods improve subtype classification. These approaches detect DUX4-r in around 5-10% of B-ALL cases.[1]

### *Short-read whole genome sequencing*

Short-read whole genome sequencing uses tools such as GRIDSS and Pelops to detect DUX4 fusion junctions despite repetitive genomic regions. Pelops successfully identified and validated DUX4-r cases in pediatric B-ALL studies.[1]

### *Long-read sequencing*

Long-read sequencing methods, including PacBio SMRT sequencing and Oxford Nanopore Technology (ONT), allow precise mapping of DUX4 fusion events across repetitive regions. These methods accurately identify IGH::DUX4 rearrangements and associated ERG deletions. However, they are costly.[1]

### *Indirect methods*

Indirect detection methods use surrogate markers such as ERG deletions and ERG<sup>alt</sup> expression, detected by MLPA, SNP arrays, or PCR. However, their reliability is limited because these markers are not present in all DUX4-r cases.[1]

### *SNV-based amplicon sequencing*

SNV-based amplicon sequencing using maternal cell-free DNA (cfDNA) and multiplex PCR enables non-invasive prenatal diagnosis of FSHD1. In a prospective study of 13 high-risk families, the method showed 100% concordance with invasive testing and provided results within 7 days.[15]

### *Optical genomic mapping (OGM)*

Optical genomic mapping (OGM) is a newer technique that can simultaneously detect D4Z4 repeat contractions and 4q haplotypes, making it simpler and more convenient than Southern blotting. OGM-based testing is currently available in India at institutions such as the CSIR Centre for Cellular and Molecular Biology.[15]

### *Flow Cytometry–Based Diagnosis*

#### *CD371 flow cytometry*

Flow cytometric detection of CD371 is a rapid, inexpensive, and highly reliable method for identifying DUX4-r B-ALL. Most DUX4-r cases express CD371, giving the method high sensitivity and specificity, especially in settings without access to sequencing technologies.[1]

### *Imaging-Based Diagnostics*

#### *MRI diagnosis*

MRI studies have identified characteristic muscle involvement patterns useful for diagnosing FSHD.

Trapezius involvement with bilateral sparing of subscapularis muscles showed high diagnostic accuracy, while additional asymmetric limb involvement was considered nearly pathognomonic for FSHD.[10]

#### *4D-CT (dynamic CT)*

4D-CT enables real-time assessment of joint and musculoskeletal movement. Although mainly used in orthopedics, it may become useful in FSHD for evaluating abnormal biomechanics, scapular instability, and gait abnormalities caused by progressive muscle weakness.[11]

#### *POCUS (Point-of-Care Ultrasound)*

POCUS provides rapid, inexpensive, radiation-free imaging and has high accuracy for evaluating soft tissue and musculoskeletal abnormalities. It may become useful for bedside assessment of muscle wasting, fatty replacement, and asymmetric muscle involvement in FSHD.[11]

### *Artificial Intelligence–Assisted Diagnostics*

#### *Artificial intelligence (AI)*

Deep learning–based analysis of whole-body MRI has shown promise in differentiating FSHD from inflammatory myopathies. AI models achieved diagnostic performance comparable to expert musculoskeletal radiologists while reducing observer variability and manual image grading.[12]

#### *Video-based biomechanical analysis*

Smartphone video–based motion analysis using the OpenCap platform quantified movement abnormalities in FSHD patients. This method showed excellent reproducibility and outperformed traditional timed function tests in identifying disease-specific movement patterns.[16]

### *Biomarkers*

#### *Ferroptosis biomarkers*

Recent studies identified increased ferroptosis-related biomarkers in FSHD muscle tissue, correlating with inflammation and fatty infiltration. Ferroptosis inhibitors and iron chelators improved membrane repair in FSHD myoblasts, suggesting a potential therapeutic target.[9]

ii. PAX7 biomarker:

Repression of PAX7 target genes was found to correlate strongly with FSHD disease severity and muscle pathology. A blood-based “FSHD muscle-blood biomarker” consisting of 143 PAX7 target genes was developed for minimally invasive disease monitoring.[13]

### *Plasma biomarker KHDC1L*

KHDC1L has been identified as a circulating plasma biomarker reflecting DUX4 activity in FSHD. Elevated plasma KHDC1L levels in FSHD patients suggest its potential role in disease monitoring and assessment of treatment response.[18]

### *Electrophysiological biomarkers*

Muscle velocity recovery cycle (MVRC) studies demonstrated altered muscle membrane excitability in FSHD patients. These electrophysiological abnormalities may serve as early biomarkers for disease monitoring and future clinical trials.[17]

### *SNV-based amplicon sequencing*

Maternal cfDNA screening with multiplex PCR-based haplotype analysis achieved 100% concordance with invasive prenatal testing in high-risk FSHD1 pregnancies.[15]

### *Plasma biomarker KHDC1L*

Elevated plasma KHDC1L levels reflected systemic DUX4 activity and showed promise as a minimally invasive biomarker for monitoring disease progression and therapeutic response.[18]

## *Advancements in Treatment of FSHD*

### *DUX4-Targeted Therapies*

Therapies directly targeting DUX4 remain the most promising disease-modifying approaches in FSHD. Losmapimod showed reduction of DUX4-regulated genes and slower MRI fat replacement in selected patients, although the later Phase III REACH trial failed to demonstrate significant clinical benefit [19-21]. RNA-targeting approaches including antisense oligonucleotides, siRNAs, miRNAs, PMOs, and CRISPR-based therapies demonstrated marked suppression of DUX4 expression and reduced muscle toxicity in preclinical models [2,4,6,7,27-32]. Newer agents such as AOC 1020, ARO-DUX4, DYNE302, MC-DX4, and EPI-321 have further improved targeted delivery and represent important emerging therapeutic candidates [34-41].

### *Muscle-Enhancing and Supportive Therapies*

Several therapies aimed at improving muscle mass and reducing muscle damage have also been investigated in FSHD. Albuterol demonstrated modest increases in lean body mass and limited strength improvement, although functional benefits remained inconsistent [3,5,22]. Antioxidant therapies such as vitamin C, vitamin E, selenium, zinc, and tempol reduced oxidative stress and

DUX4-associated cellular injury, but clinical benefits were generally modest [4,5,7,24]. Myostatin inhibitors including MYO-029 and ACE-083 increased muscle volume but failed to consistently improve functional outcomes [5,25]. Newer anti-myostatin agents such as RO7204239 and regenerative therapies like SAT-3247 continue to be evaluated in ongoing studies [26,41].

### *Emerging Small-Molecule and Anti-Inflammatory Approaches*

Novel small-molecule therapies targeting downstream disease pathways are also being explored. G-quadruplex ligands such as berberine and PhenDC3 suppressed DUX4 expression and reduced fibrosis in experimental models [4,32]. The hyaluronic acid inhibitor 4-methylumbelliferone improved mitochondrial abnormalities and cellular morphology associated with DUX4 activity [4,33]. Inflammatory pathways have also emerged as therapeutic targets, with elevated IL-6 levels identified in FSHD patients. This led to the development of satralizumab, an anti-IL-6 monoclonal antibody currently being evaluated to reduce inflammation and disease progression [26].

### *Observational Studies and Disease Monitoring*

Observational studies and registries are essential for understanding disease progression and improving future clinical trials in FSHD. Major studies such as ReSolve, ADVANCED, WANTED, and PROGRESS-FSHD are evaluating MRI biomarkers, wearable monitoring systems, and digital outcome measures to better assess disease activity. National registries in France, the United States, the United Kingdom, and China are providing valuable longitudinal clinical and genetic data for epidemiological research and patient recruitment. Additional studies such as INSIGHT-FSHD2 are investigating inflammatory biomarkers, pulmonary involvement, and functional outcome measures to improve long-term disease monitoring.

Strength of current evidence: The evidence reviewed in this study spans multiple levels of the evidence hierarchy. Molecular diagnostic studies and imaging biomarkers are primarily supported by observational cohort studies with external validation. Most RNA-targeted therapies, CRISPR-based interventions, antisense oligonucleotides, and gene-editing strategies remain at the preclinical stage, with efficacy demonstrated mainly in cellular and animal models. Clinical evidence is currently strongest for losmapimod and muscle-enhancing agents, although randomized trials have generally failed to demonstrate

meaningful clinical benefit. Registry studies and natural history cohorts provide valuable information regarding disease progression and outcome measures but do not establish therapeutic efficacy. Likewise, many therapeutic strategies remain limited by small sample sizes, short follow-up periods, surrogate outcome measures, and uncertainty regarding long-term safety. Consequently, several interventions should currently be regarded as experimental rather than established therapies.

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