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REVIEW ARTICLE

Advances in Biomarkers for Muscular Dystrophies: a Comprehensive Review

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ABSTRACT

Background: **Muscular dystrophies (MDs) are a group of genetically heterogeneous diseases characterized by progressive muscle degeneration and functional impairment. Current treatment options remain limited, highlighting the urgent need for biomarkers to enhance diagnosis, monitor disease progression, and evaluate treatment efficacy across different subtypes, such as Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), facioscapulohumeral muscular dystrophy (FSHD), and limb-girdle muscular dystrophy (LGMD).**
Methods: **A search of the MEDLINE/PubMed databases was conducted for original and review articles that examined biomarkers associated with muscular dystrophies. Based on the findings from these articles, a comprehensive review was constructed.**
Results: **The study identified significant advancements in various categories of biomarkers, including protein biomarkers, microRNA profiles, imaging parameters, functional assessments, as well as emerging fields such as extracellular vesicles and epigenetic modifications.**
Conclusions: **Research on biomarkers in muscular dystrophies has evolved from a focus on single biomarkers to a comprehensive multi-omics approach. Future research directions should emphasize multicenter validation and the development of biomarker-guided therapeutic strategies to advance the application of personalized medicine in muscular dystrophies.**
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INTRODUCTION

Muscular dystrophies (MDs) encompass a heterogeneous group of inherited neuromuscular disorders characterized by progressive skeletal muscle degeneration, weakness, and functional impairment, significantly affecting patients' quality of life and longevity [1-12]. These conditions typically involve defects in structural proteins that are critical for maintaining muscle integrity, leading to recurrent cycles of muscle fiber damage, inflammation, and eventual replacement of muscle tissue with fibrotic and adipose tissue. The clinical spectrum of MDs ranges from severe, early-onset forms to milder variants that present later in life, with Duchenne muscular dystrophy (DMD) being the most common

and severe subtype. DMD results from a deficiency of dystrophin, which causes progressive muscle wasting and life-threatening cardiorespiratory complications. In contrast, Becker muscular dystrophy (BMD) exhibits a milder phenotype due to the presence of partially functional dystrophin. Other forms, such as facioscapulohumeral dystrophy (FSHD), limb-girdle muscular dystrophy (LGMD), and myotonic dystrophy type 1 (DM1), display distinct genetic mechanisms and clinical presentations, including asymmetric weakness in FSHD and progressive girdle muscle involvement in LGMD.

Despite decades of research, effective disease-modifying therapies for most MDs remain limited, with current treatments, such as glucocorticoids, offering symptomatic relief but often accompanied by significant side effects [13,14]. This therapeutic gap underscores the urgent need for sensitive and specific biomarkers to enhance diagnosis, monitor disease progression, and evaluate therapeutic efficacy. Recent technological advances have revolutionized biomarker discovery, allowing for the comprehensive characterization of molecular signatures through multi-omics approaches [15,16], advanced imaging modalities [4,5,11], and innovative functional assessments [17]. Biomarker research has expanded to include a diverse array of categories [18-39], such as protein biomarkers [7,23], microRNA signatures [26,27], extracellular vesicles [28], and epigenetic modifiers [9,29,30].

This review aims to systematically synthesize recent advances in biomarkers for MDs, drawing upon evidence from recent studies across various MD subtypes. It will critically examine the current landscape of biomarker research, address ongoing challenges such as standardization and validation, and explore future directions for implementing biomarker-driven strategies in clinical practice and therapeutic development.

Protein biomarkers

Significant progress has been made in identifying and characterizing protein biomarkers across various forms of muscular dystrophy, providing valuable insights into disease mechanisms and potential tools for monitoring disease progression and therapeutic response.

In Duchenne and Becker muscular dystrophies (DMD/BMD), serum proteomic analysis has uncovered several proteins with differential expression. A study utilizing Data Independent Acquisition Mass Spectrometry identified proteins such as Creatine Kinase M-type (CKM), Pyruvate Kinase (PKM), Fibrinogen Gamma Chain (FGG), Lactate Dehydrogenase B (LDHB), and Alpha-2-Macroglobulin (A2M) as having altered longitudinal signatures between DMD and BMD. These findings suggest roles for these proteins in immune response, extracellular matrix organization, and hemostasis, extending beyond mere muscle leakage [7]. Additionally, Urinary Titin, a degradation product of the giant sarcomeric protein, has been established as a biomarker in BMD, with elevated levels correlating with loss of ambulation, reduced muscle strength, and increased markers of skeletal and cardiac damage, such as serum creatine kinase and cardiac troponin I [23]. The alarmin High Mobility Group Box 1 (HMGB1) protein has also emerged as a candidate biomarker, found to be elevated in a human iPSC-derived skeletal myocyte model of DMD and in the B10-mdx mouse model. Its reduction following microdystrophin treatment suggests its potential as a biomarker for monitoring inflammatory pathology and treatment efficacy [6]. Furthermore, a multi-omics meta-analysis of DMD muscle tissue has highlighted hub genes involved in extracellular matrix remodeling, including COL6A3, COL1A1, COL3A1, COL1A2, POSTN, TIMP1, THBS2, and SPP1, which are central to the protein interaction network associated with the disease [16]. However, the interpretation of Cardiac Troponin T (cTnT) levels in muscular dystrophy patients requires caution, as elevations can occur in the absence of acute coronary events, potentially leading to misinterpretations of cardiac status in this population [31].

In Facioscapulohumeral Dystrophy (FSHD), inflammatory pathways are highlighted by elevated circulating cytokines. Serum levels of IL-6 and TNF are significantly higher in FSHD patients compared to healthy controls. These cytokines are also produced at higher levels by ex vivo stimulated NK cells and are present in muscle specimens, with IL-6 levels showing a mild correlation with disease duration, disease severity, and muscle weakness [10].

For Oculopharyngeal Muscular Dystrophy (OPMD), a plasma proteomic study identified a distinct signature associated with disease severity. Proteins such as ACTN2, MYOM2, CA3, APOBEC2, MYL3, and PDLIM3 were markedly elevated (over 200%) in patients with limb weakness compared to those without and healthy controls. These proteins demonstrated a strong correlation with the degree of fatty replacement in the soleus muscle, making them promising biomarkers for disease progression and prognosis [32].

In Dysferlinopathy, comprehensive proteomic profiling of muscle tissue has linked the expression of specific proteins to the extent of histopathological changes. Proteins such as ANXA1, ANXA2, ANXA4, ANXA5, LMNA, and PYGM were identified, and their expression levels were validated to correlate with the dystrophic pathology observed in muscle biopsies, providing new potential targets for therapeutic endeavors [33].

In summary, the landscape of protein biomarkers in muscular dystrophy is expanding, encompassing markers of muscle damage, inflammation, extracellular matrix remodeling, and specific pathological processes across different dystrophies. These biomarkers hold considerable promise for improving disease stratification, monitoring progression, and evaluating the efficacy of emerging therapies.

microRNA biomarkers

microRNAs (miRNAs) have emerged as promising biomarkers in muscular dystrophy due to their stability in

biofluids, muscle-specific expression patterns, and roles in regulating key pathological processes. Recent research has focused on identifying both general markers of muscle damage and disease-specific miRNA signatures.

Muscle-enriched miRNAs, known as myomiRs, demonstrate significant potential as general biomarkers for muscle damage across various dystrophies. As extensively reviewed in the context of Limb-Girdle Muscular Dystrophy (LGMD), miRNAs such as miR-1, miR-133a/b, and miR-206 are frequently dysregulated. Notably, circulating levels of miR-206 are significantly elevated in LGMD patients compared to healthy subjects, suggesting its potential as a general biomarker of muscle damage across various muscular dystrophies. Research also indicates the possibility of developing subtype-specific molecular signatures; for instance, specific miRNA combinations might help discriminate between different LGMD subtypes such as LGMDR1, LGMDR2, LGMDR3, and LGMDR4 [35]. However, the reliable quantification of these miRNAs depends on the use of appropriate reference miRNAs for normalization. A study in the DE50-MD canine model of DMD identified miR-191, let-7b, miR-125a, and miR-15a as the most stable reference miRNAs for normalizing expression data across different ages and muscle types, outperforming the commonly used U6 snRNA. When normalized to this panel, dystrophic muscle showed lower expression of miR-1 and miR-133a, while the "fibromiR" miR-214 was found to be 2- to 4-fold higher in dystrophic versus wild-type muscles [26].

Beyond general muscle markers, specific miRNA pathways are being unraveled in particular dystrophies. In Myotonic Dystrophy Type 1 (DM1), a novel regulatory axis involving a circular RNA and a miRNA has been discovered. The circular RNA circARHGAP10 is significantly increased in DM1 muscle biopsies, and its expression correlates positively with CTG repeat length and inversely with muscle strength, indicating its potential as a biomarker. Mechanistic studies revealed that circARHGAP10 binds to and sequesters miR-409-3p. Both were found to be upregulated in DM1, and silencing of circARHGAP10 led to a downregulation of miR-409-3p. Importantly, the beneficial effects of circARHGAP10 silencing on reducing nuclear foci and rescuing splicing defects were blocked by miR-409-3p overexpression, indicating that the pathogenic role of circARHGAP10 is mediated, at least in part, through its interaction with miR-409-3p. This positions the circARHGAP10/miR-409-3p axis as a promising biomarker and therapeutic target for DM1 [27].

Overall, miRNA biomarkers offer a versatile toolset for muscular dystrophy. They range from general indicators of muscle pathology, such as miR-206, to intricate disease-specific regulatory networks, as seen in DM1 with circARHGAP10 and miR-409-3p.

The ongoing refinement of normalization strategies and the discovery of such specific pathways significantly advance our potential for precise molecular diagnosis, disease monitoring, and the development of targeted therapies.

Genetic & epigenetic modifiers

The landscape of biomarkers in muscular dystrophy has expanded beyond traditional proteins to include genetic and epigenetic modifiers, which provide profound insights into disease mechanisms, enhance diagnostic precision, and open new avenues for therapeutic targeting. These biomarkers often reflect the fundamental genetic lesions or the consequent epigenetic dysregulation that drives pathology.

In Facioscapulohumeral Dystrophy (FSHD), the quintessential epigenetic biomarker is the hypomethylation of the D4Z4 macrosatellite repeat on chromosome 4q35. This loss of repressive chromatin marks leads to the aberrant expression of the DUX4 gene, which is toxic to muscle cells. Analysis of DNA methylation at specific regions within the locus (DUX4-PAS and DR1) has been refined into a highly effective clinical diagnostic tool. Importantly, interpreting the methylation profile must consider the patient's 4q/10q haplotype background (e.g., 4qA/4qA vs. 4qA/4qB), as distinct methylation thresholds are required for accurate classification in these different genetic contexts. This assay demonstrates high sensitivity and specificity in identifying patients with FSHD and can even detect hypomethylated profiles in asymptomatic individuals, suggesting its potential utility in identifying preclinical cases, especially those with a positive family history [29].

Myotonic Dystrophy Type 1 (DM1), caused by a CTG repeat expansion, exhibits a complex pathomechanism involving RNA toxicity and splicing defects. Beyond the primary genetic defect, downstream epigenetic players are emerging as biomarkers. The circular RNA circARHGAP10 is significantly upregulated in DM1 muscle tissue. Its expression level correlates positively with the CTG repeat length and inversely with muscle strength, positioning it as a promising biomarker for disease severity. Furthermore, its function is mechanistically linked to the sequestration of miR-409-3p, which is also upregulated in DM1. This regulatory axis represents a novel layer of epigenetic regulation and a potential therapeutic target, as modulating this circuit can ameliorate molecular defects in cellular models [27].

Another approach directly targets the core splicing pathology. The functional depletion of Muscleblind-like (MBNL) proteins is a key trigger of DM-associated alternative splicing defects. A novel strategy using small activating RNAs (saRNAs) targeted to the MBNL1 promoter can stimulate its transcription. This site-specific augmentation of endogenous MBNL1 expression has been shown to correct the alternative splicing of multiple biomarker exons in DM1 cell models, offering a new therapeutic strategy and a clear biomarker of target engagement and efficacy [30].

In conclusion, genetic and epigenetic modifiers represent a powerful and sophisticated class of biomarkers in muscular dystrophy. They range from direct measures

Table 1. Molecular biomarkers for muscular dystrophies.

Molecular biomarker type	Specific biomarker	Associated Disease	Reference
Protein biomarker	HMGB1	DMD	[6]
Protein biomarker	CKM, PKM, FGG, LDHB, A2M	DMD, BMD	[7]
Protein biomarker	IL-6, TNF	FSHD	[10]
Protein biomarker	COL6A3, COL1A1, COL3A1, COL1A2, POSTN, TIMP1, THBS2, SPP1	DMD	[16]
Protein biomarker	Urinary titin	BMD	[23]
Protein biomarker	Cardiac troponin T (cTnT)	MDs	[31]
Protein biomarker	ACTN2, MYOM2, CA3, APOBEC2, MYL3, PDLIM3	OPMD	[32]
Protein biomarker	ANXA1, ANXA2, ANXA4, ANXA5, LMNA, PYGM	dysferlinopathy	[33]
microRNA biomarker	miR-214	DMD	[26]
microRNA biomarker	miR-1, miR-133a/b, miR-206	LGMD, DMD	[26,35]
microRNA biomarker	miR-409-3p	DM1	[27]
Genetic & epigenetic modifier	D4Z4 methylation (DUX4-PAS, DR1)	FSHD	[9,29]
Genetic & epigenetic modifier	circARHGAP10	DM1	[27]
Genetic & epigenetic modifier	MBNL1 splicing defects	DM1	[30]

Table 2. Imaging biomarkers for muscular dystrophies.

Imaging biomarker type	Specific biomarker	Associated disease	Reference
Ultrasound	Attenuation imaging (RRFM method)	DMD	[5]
Ultrasound	Diaphragm ultrasound (VVS, ECHO-MRR)	FSHD	[36]
Magnetic Resonance Imaging (MRI)	Oxygenation-sensitive MRI (OS-CMR)	DMD	[4]
Magnetic Resonance Imaging (MRI)	Quantitative MRI (fat fraction, water T2, water T1, pH)	LGMD-R9	[11]
Magnetic Resonance Imaging (MRI)	MRI fat fraction (Gluteus Maximus)	Pediatric BMD	[12]

Table 3. Functional biomarkers for muscular dystrophies.

Functional biomarker type	Specific Biomarker	Associated Disease	Reference
Video-based biomechanics	Movement signatures (via OpenCap)	FSHD, DM1	[17]
Ambulatory test	6-Minute Walk Test (6MWT)	DMD (canine model)	[37]
Muscle excitability	Muscle Velocity Recovery Cycles (MVRC)	FSHD	[38]
Kinematic analysis	Escape response kinematics	DMD (zebrafish model)	[39]

of primary epigenetic defects, such as D4Z4 hypomethylation in FSHD, and to downstream RNA-based regulatory circuits and splicing-based outcomes in DM1. These biomarkers are revolutionizing diagnostics by providing a molecular level of precision, are essential for defining preclinical disease states, and are increasingly serving as direct measures of response in emerging therapeutic trials aimed at modulating gene expression and correcting epigenetic dysregulation.

Imaging biomarkers

Imaging biomarkers have become indispensable tools in the clinical management and therapeutic development for muscular dystrophies, offering non-invasive, quantitative, and sensitive measures of disease involvement across muscle groups and organ systems. Recent advancements have refined these techniques to provide highly specific pathophysiological insights.

Quantitative magnetic resonance imaging (qMRI) is used to assess skeletal muscle pathology in Limb-Girdle Muscular Dystrophy R9 (LGMD-R9). Baseline measurements of muscle fat fraction (FF), water T2, and water T1 are significantly elevated in patients compared to healthy controls. Notably, baseline water T2 and water T1 values show a strong positive correlation with the increase in FF and functional decline over two years, suggesting their potential for predicting disease progression [11]. In pediatric Becker Muscular Dystrophy (BMD), where clinical heterogeneity poses a challenge, qMRI effectively detects early muscle involvement. Among various parameters and muscle sites, the fat fraction in the gluteus maximus demonstrates the best diagnostic performance. It exhibits the strongest negative correlation with the North Star Ambulatory Assessment (NSAA) score and emerges as an independent risk factor for predicting functional decline, establishing it as a preferred biomarker for this population [12].

Ultrasound technologies have also evolved, providing dynamic and accessible biomarkers. For Duchenne Muscular Dystrophy (DMD), quantitative ultrasound has been extended beyond envelope statistics to attenuation imaging. The development of the robust reference frequency method (RRFM) significantly reduces measurement bias. When applied to the gastrocnemius muscle, RRFM-based attenuation imaging demonstrates an outstanding ability to discriminate between early and late ambulatory stages in DMD patients, outperforming other quantitative ultrasound (QUS) envelope statistics and offering a sensitive tool for monitoring functional deterioration [5]. In Facioscapulohumeral Dystrophy (FSHD), diaphragmatic assessment is crucial, yet facial weakness can compromise standard pulmonary function tests. Diaphragm ultrasound offers a solution, with parameters such as diaphragm excursion velocity (VVS) and maximal relaxation rate (ECHO-MRR) of the right hemidiaphragm showing significant differences between FSHD patients with and without a restrictive ventilatory defect. These parameters are independently associated with disease severity, including early lower extremity involvement and higher clinical severity scores, positioning them as informative biomarkers for respiratory involvement and overall disease burden [36].

Beyond skeletal muscle, cardiac-specific imaging biomarkers are gaining prominence in dystrophies with cardiac involvement. In DMD-associated cardiomyopathy, oxygenation-sensitive cardiac magnetic resonance (OS-CMR) reveals the presence of myocardial hypoxia, a previously understudied aspect. Myocardial oxygenation signal intensity is significantly decreased in DMD patients compared to controls, and this reduction is more pronounced in segments with late gadolinium enhancement (LGE), indicating fibrosis. The inferolateral wall oxygenation was identified as an independent predictor of LGE presence, suggesting that myocardial hypoxia evaluated by OS-CMR is an early event in DMD cardiomyopathy and a potential biomarker for assessing myocardial damage prior to overt fibrosis [4].

In summary, the field of imaging biomarkers is rapidly advancing toward more precise, pathophysiologically informed, and multi-systemic assessments. From qMRI and ultrasound of skeletal and respiratory muscles to specialized cardiac MR techniques, these modalities provide an objective, quantitative, and comprehensive picture of disease burden and progression. Their integration into clinical practice and trials is essential for improving patient monitoring and robustly evaluating the efficacy of emerging therapies.

Functional biomarkers

Functional biomarkers play a critical role in assessing disease progression, monitoring therapeutic efficacy, and capturing real-world motor impairments in muscular dystrophies. These biomarkers encompass a range of performance-based tests and advanced analytical methods that provide objective, quantifiable measures of physical function, often complementing molecular and imaging approaches. Recent advances have focused on refining traditional ambulatory tests and introducing innovative technologies to enhance sensitivity and disease specificity.

Ambulatory tests, such as the 6-minute walk test (6MWT), remain a cornerstone for evaluating functional capacity in both clinical and pre-clinical settings. In the DE50-MD canine model of Duchenne muscular dystrophy (DMD), the 6MWT effectively discriminates between dystrophic and wild-type dogs from as early as 3 to 18 months of age, with no significant difference observed between repeated tests conducted 48 hours apart. This test's reliability and its ability to reflect disease severity without altering stable blood-borne biomarkers like CCL2, MYOM3, and MSTN make it a robust functional outcome measure for interventional studies [37].

Beyond traditional timed function tests, video-based biomechanical analysis has emerged as a powerful tool for capturing disease-specific movement signatures with high precision. Using open-source platforms like OpenCap, researchers can quantify 3D movement kinematics from smartphone videos in a median time of just 16 minutes per participant. This approach not only reproduces standard timed function tests (e.g., 10-meter walk, timed up-and-go) with high correlation ($r > 0.98$) but also identifies subtle, disease-specific patterns in conditions such as facioscapulohumeral dystrophy (FSHD) and myotonic dystrophy type 1 (DM1). For example, video-derived features outperform timed tests in classifying disease status and reveal kinematic differences in gait that are not evident through conventional

measures, offering a more sensitive and comprehensive assessment of motor function [17].

Muscle excitability parameters, assessed through muscle velocity recovery cycles (MVRC), provide insights into sarcolemmal dysfunction in disorders like FSHD. Recordings from the tibialis anterior and trapezius muscles in FSHD patients show significantly reduced early and late supernormality compared to healthy controls, indicating resting membrane potential depolarization. Although correlations with clinical severity scores are moderate, these electrophysiological measures serve as early biomarkers of membrane instability, potentially preceding overt structural changes [38].

In animal models, functional biomarkers are being refined to improve translational relevance. For instance, in a zebrafish model of DMD, escape response kinematics analyzed via high-speed videography and deep learning-based motion capture identify precise biomarkers of the dystrophic phenotype. Parameters such as overall escape distance, peak speed, and tail kinematics (e.g., angular velocity) differentiate mutant from wild-type larvae with large effect sizes. These kinematic measures act as non-lethal proxies for skeletal muscle dysfunction and reveal mechanistic insights into mobility deficits, such as impaired temporal aspects of tail movements, which correlate with in vitro contractility assessments [39].

To summarize, functional biomarkers are evolving from simple timed tests to sophisticated, multi-dimensional assessments that capture the complexity of neuromuscular diseases. The integration of ambulatory measures, biomechanical analyses, electrophysiological techniques, and model system evaluations provides a holistic view of functional decline and therapeutic response. These advancements not only enhance clinical trial design but also pave the way for personalized management strategies in muscular dystrophy.

Emerging biomarkers

The field of muscular dystrophy biomarker research is rapidly expanding beyond traditional blood and imaging markers to include novel, highly sensitive molecular and cellular indicators. These emerging biomarkers, often derived from cutting-edge technological platforms, offer unprecedented insights into disease-specific pathophysiological processes and hold significant promise for non-invasive monitoring and therapeutic development.

One of the most promising frontiers is the analysis of extracellular vesicles (EVs)-membrane-bound particles secreted by cells. EVs are commonly categorized into exosomes, microvesicles, and apoptotic bodies according to their size, biogenesis, and function. By transporting cargoes such as lipids, proteins, and RNAs-particularly microRNAs-EVs facilitate intercellular communication and are thought to contribute to key pathological processes in Duchenne muscular dystrophy (DMD), including muscle degeneration, fibrosis, inflammation, and cardiomyopathy. Given their functional roles, EVs are increasingly regarded not only as potential biomarkers for disease monitoring, but also as promising vehicles for novel therapies [22].

The role of EVs extends beyond diagnostics into the realm of intercellular communication and pathophysiology. A comprehensive review highlights that exosomes, a subtype of EVs, mediate crucial crosstalk between the cardiovascular and musculoskeletal systems. These vesicles contain bioactive substances, including microRNAs, long non-coding RNAs, lipids, and proteins. Under pathological conditions such as muscular dystrophy, the cargo of muscle-derived exosomes is altered, potentially contributing to secondary complications like cardiomyopathy. For instance, skeletal muscle-derived exosomes may protect cardiomyocytes through the delivery of functional molecules, but in states of severe wasting, they might also transmit pro-apoptotic signals that exacerbate cardiac dysfunction. This intricate bidirectional communication establishes exosomes and their specific cargo not only as biomarkers reflecting systemic disease activity but also as novel therapeutic targets and even as engineered drug delivery vehicles to mitigate multi-organ pathology in muscular dystrophies [28].

Overall, emerging biomarkers like EVs and exosomes represent a paradigm shift in muscular dystrophy research. These advancements highlight the movement toward highly personalized, mechanism-based biomarkers that could transform patient management in the future.

DISCUSSION

In this study, we reveal significant advancements in biomarker research for muscular dystrophies (MDs) while concurrently highlighting critical challenges and future directions. The field has evolved considerably beyond single-molecule biomarkers, embracing multi-parametric and multi-modal approaches that provide a more holistic view of disease pathology and progression. Research has demonstrated the value of integrating diverse biomarker classes for comprehensive disease monitoring. Multi-omics approaches have identified numerous protein [7,16,33] and miRNA [26,27,35] biomarkers that reflect various aspects of MD pathophysiology. Advanced imaging techniques, including quantitative MRI [11,12] and ultrasound methodologies [5,36], have provided non-invasive tools for quantifying muscle pathology and respiratory function. Additionally, epigenetic markers, particularly D4Z4 methylation in facioscapulohumeral dystrophy (FSHD) [29], have emerged as valuable diagnostic tools. Novel biomarker sources such as extracellular vesicles [22,28] and innovative functional assessments using video-based biomechanics [17] offer promising avenues for sensitive disease monitoring.

Despite these advancements, several significant challenges remain. Standardization across studies and centers is lacking, with methodological variations contributing to inconsistent findings [9]. Patient heterogeneity within and between MD subtypes presents obstacles for

biomarker validation and application [7]. Many promising biomarkers require validation in larger, multi-center cohorts to establish their clinical utility. The complexity of MD pathologies necessitates combinatorial biomarker approaches rather than reliance on single markers. Furthermore, the translation of biomarkers from discovery to clinical application faces numerous technical and regulatory hurdles.

Based on the current state of research, several key directions emerge as priorities for future exploration. First, multi-omics integration-combining genomic, proteomic, metabolomic, and transcriptomic data-will provide deeper insights into disease mechanisms and facilitate the identification of robust biomarker panels [15,16]. Second, artificial intelligence and machine learning technologies should be leveraged to analyze complex biomarker data, identify patterns, and develop predictive models for disease progression and treatment response [17]. Additionally, establishing large, diverse patient registries and standardizing biomarker measurement protocols across centers are essential for advancing the field [9]. A focus on preclinical and early disease detection is also crucial, with an emphasis on biomarkers that enable early diagnosis and intervention, particularly in presymptomatic stages.

In conclusion, while significant progress has been made in MD biomarker research, the translation of these advances into clinical practice requires addressing current limitations through collaborative, multidisciplinary efforts. The continued development and validation of robust biomarkers will be essential for advancing personalized medicine approaches and improving outcomes for individuals with muscular dystrophies.

Declaration of Generative AI in Scientific Writing:

The author declares that no generative AI tools were used in any part of the research process or the preparation of this manuscript.

Declaration of Interest:

The author declares that there are no conflicts of interest.

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