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Genetic Modifiers of Duchenne and Facioscapulohumeral Muscular Dystrophies

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Abstract

Muscular dystrophy is defined as the progressive wasting of skeletal muscles that is caused by inherited or spontaneous genetic mutations. Next-generation sequencing (NGS) has greatly improved the accuracy and speed of diagnosis for different types of muscular dystrophy. Advancements in depth of coverage, convenience, and overall reduced cost, have led to the identification of genetic modifiers that are responsible for phenotypic variability in affected patients. These genetic modifiers have been postulated to explain key differences in disease phenotypes including age of loss of ambulation, steroid-responsiveness, and the presence or absence of cardiac defects in patients with the same form of muscular dystrophy. Here we review and highlight recent findings on genetic modifiers of Duchenne and Facioscapulohumeral muscular dystrophies based on animal and clinical studies. These genetic modifiers hold great promise to be developed into novel therapeutic targets for the treatment of muscular dystrophies.

Keywords

muscular dystrophy; genetic modifier; variant; muscle disease; DMD

Introduction

Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy worldwide with estimates of incidence ranging from 1:3500 to more recent estimates of

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1:5000 live male births(1, 2). The genetic cause of DMD was identified in 1986 as pathogenic loss-of-function mutations in the dystrophin (*DMD*) gene that lead to insufficient, and sometimes undetectable, levels of a functional dystrophin protein(3). Conversely, in Becker muscular dystrophy (BMD) a partially-functional, truncated dystrophin protein is produced, typically resulting in a milder clinical pathology(3–6). DMD patients have severe, progressive skeletal muscle wasting and cardiac defects. Many DMD patients lose ambulation by their first decade of life(7). An interesting yet understudied aspect of DMD pathology is that approximately 20–25% of DMD boys develop significant cognitive issues that fall into the Autism spectrum disorder (ASD) scale(7, 8). One explanation for this variation in DMD boys is the alteration in expression levels of neuronal dystrophin protein isoforms, although it remains unclear as to why some DMD boys develop cognitive impairment and others have normal intelligence(9–13). As biochemical and molecular techniques for muscular dystrophy diagnostics improved, additional Dystrophin-interacting proteins were identified(14–16). These proteins are part of a Dystrophin-associated protein complex (DAPC) that bridges the actin cytoskeleton with the extracellular matrix (ECM)(17). With the advancement in genomic sequencing and coverage of the human genome, improved gene panels shifted muscular dystrophy diagnostic methods from predominantly pathology/histology-based to Sanger sequencing and later whole exome/genome sequencing (WES/WGS)(18–24).

The sequencing of increasingly larger numbers of exomes and genomes from healthy and diseased individuals permits large-scale genetic analyses of modifiers of diseases. The Exome Aggregation Consortium (ExAC) and 100,000 genomes project (UK) have banked large datasets of publically available genomic information(25, 26). Genomic analyses of children with Mendelian diseases have revealed that genetic modifiers of diseases have an incomplete penetrance of disease symptoms and pathogenicity(27). Indeed, many predicted loss-of-function (LoF) pathogenic mutations have been identified in healthy children and adults (“human knockouts”), suggesting an incomplete disease penetrance due to protective genetic modifiers(28, 29). As genomic sequencing has become more commonplace, the ability to analyze large amounts of data from different populations of healthy and diseased patients has become easier. Large scale databases and registries have been established to better share genomic and medical data among researchers studying muscle diseases(30, 31). These studies have yielded copious amounts of data on the wide spectrum of phenotypic variation among patients with muscular dystrophies. In conjunction with novel animal model screening platforms, these studies have identified several genetic modifiers of different forms of muscular dystrophy. Whole genomic sequencing among inbred strains of mice, such as the MRL super-healing strain which blocks dystrophic muscle pathology when mated to dystrophin-deficient *mdx* mice, have yielded valuable genetic modifiers of disease pathology(32). This review will focus on the identification and functional consequences of genetic modifiers in the two most prevalent forms of muscular dystrophy in humans: DMD and Facioscapulohumeral muscular dystrophy (FSHD). We also highlight current advancements in the clinic for these diseases and how genetic modifiers identified from both patient studies and animal models have led to novel therapeutic targets that may affect disease outcomes. These genetic modifiers hold the potential for ameliorating muscular dystrophies, and offer hope for patients with these debilitating diseases.

Genetic modifiers of Duchenne muscular dystrophy

A large number of the Duchenne muscular dystrophy genetic modifiers have been identified in dystrophin-deficient animal models and more recently in genomic analyses of DMD patient cohorts (Table 1). Since the discovery of the first *mdx* mouse arising from a spontaneous mutation in a mouse colony from the United Kingdom, many additional dystrophin-deficient vertebrate animal models have been generated and phenotypically evaluated(33–36). *Mdx* mice display progressive skeletal muscle weakness but do not share the same reduced lifespan and early death as observed in DMD patients. This is thought to be due to increased expression of the dystrophin protein analog utrophin, which is expressed during embryonic myogenesis but is silenced during adult myogenesis(37, 38) (Figure 1). An alternative explanation for this phenomenon may be the increased presence in *mdx* muscle of revertant (dystrophin-positive) myofibers, resulting from undefined RNA-splicing or naturally occurring exon-skipping mechanisms(39–41). Exogenous overexpression of utrophin or compounds that can activate utrophin expression in skeletal muscle remains a promising therapeutic strategy for DMD(42). Transgenic mice that overexpress utrophin on a dystrophin-deficient background have been shown to rescue both the muscle pathology and motor deficits, thereby preventing muscular dystrophy disease progression(43). Drug compounds that induce utrophin transcriptional activation in dystrophin-deficient skeletal muscle (SMT C1100; Ezutromid) are in current clinical trials in DMD boys(44). Ezutromid induces transcription of utrophin mRNA in adult muscle where it is transcriptionally silenced, and thereby results in expression of utrophin protein to compensate for the lack of functional dystrophin protein(45).

Recent studies in *mdx* mice have also implicated additional genetic modifiers of dystrophin-deficiency that are found in inbred mouse strains that may also explain the genetic variation in both DMD mice and humans(46–48). In humans, the presence of the R577X (rs1815739) null polymorphism in the α-ACTININ-3 (*ACTN3*) gene is associated with better overall muscle endurance(49). Male and female athletic sprinters show a higher percentage of the 577R allele over non-sprinters and the general population as a whole(50). In the context of dystrophin-deficiency, the loss of *Actn3* in mice blocks muscle wasting and degeneration in *mdx* mice(51). A Golden Retriever muscular dystrophy (GRMD) dog was identified from a litter of inbred dogs and found to contain a splice acceptor variant resulting in the retention of intron 6 of the canine dystrophin gene(52, 53). Interestingly, in a Brazilian colony of GRMD dogs, an “escaper” dog named Ringo was shown to have no detectable levels of dystrophin protein, but a milder clinical phenotype, normal reproductive capabilities, and a normal lifespan(54, 55). Whole genome sequencing and RNA transcriptome profiling of the muscles from the Golden Retriever muscular dystrophy canine model and the escaper GRMD dog that had a milder phenotype revealed that increased levels of Jagged-1 (*Jag1*) improved dystrophic symptoms in both zebrafish and dog DMD models(56). With improved genomic editing using clustered regularly interspaced short palindromic repeats (CRISPR) technologies, newer DMD animal models may yield additional genetic modifiers that may lessen or worsen disease symptoms. These dystrophin-deficient models may be beneficial in identifying naturally occurring strain variants that yield additional protective or harmful genetic modifiers of dystrophin deficiency.

Several genetic modifiers of muscular dystrophies have been identified from mouse crosses and are currently being explored for therapeutic targets. Expression levels of two important genetic modifiers (α-7 integrin and laminin-α2) were shown to be elevated in mdx mice and the GRMD dog models of DMD treated with prednisone(57). Loss of α-7 integrin (*Itga7*) in mice on the *mdx* background worsens muscle weakness and increases levels of fibrosis(58, 59). Conversely, transgenic overexpression of α-7 integrin reduces muscle weakness and muscle force deficits in both *laminin-α2* and *mdx/utrn* double mutant mice(60, 61). A small molecule SU9516 has been shown to rescue muscular dystrophy phenotypes in *mdx* mice due to its ability to transcriptionally activate the α-7 integrin promoter(62). Other genetic modifiers such as biglycan (*BGN*), sarcospan (*SSPN*), and galectin-1 (*Lgals1*) overexpression have been demonstrated to act as a muscle membrane "glue" to increase muscle myofiber membrane stability and block muscle tearing in *mdx* mice(63–65) (Table 1). Biglycan functions to protect against muscle force loss by acting as a protein "anchor" to stabilize the muscle myofibers and their link to the extracellular matrix (ECM) via an interaction with the dystrophin-glycoprotein complex (DGC)(66, 67). (Figure 1). In DMD, Biglycan expression levels are slightly elevated as it is capable of interacting and sequestering TGF-β in the ECM(68, 69). Given the strong role of TGF-β for driving inflammation in DMD, TGF-β antagonists have been effective in blocking some of the dystrophic muscle symptoms associated with DMD(70, 71). Direct intravenously-administered AAV-mediated overexpression of exogenous human biglycan (*BGN*) was recently shown to ameliorate muscle grip strength deficits and improve overall histology in dystrophin-deficient mice(72). Similar to biglycan, the protein sarcospan (*SSPN*) also acts as a membrane "glue" to anchor the muscle membrane via an interaction with sarcoglycans, further stabilizing the dystrophin-glycoprotein complex(73). Transgenic overexpression of sarcospan in *mdx* mice blocks muscle pathology via increasing the levels of utrophin-glycoprotein complex and activates AKT signaling as a compensatory mechanism for the lack of Dystrophin expression(74, 75). Follow-up transgenic mouse studies demonstrated that high levels of sarcospan were sufficient to rescue cardiac and pulmonary defects in *mdx* mice(76, 77). Together these studies strongly support the notion that overexpression (either naturally-occurring genetic variants or artificially engineered) of selected membrane-associated proteins may be beneficial in blocking or ameliorating dystrophic pathology. As with all of these therapeutic compounds resulting from genetic modification or exogenous delivery, it is possible that human DMD patients may harbor protective or pathogenic variants in these genetic factors that may predict disease progression and outcomes. More whole genome studies of both healthy individuals and the DMD population are needed to test this hypothesis.

Genetic modifiers of DMD identified from study of affected patient populations

Case reports of DMD patients have revealed genetic modifiers that might explain differences in clinical severity. In one case report, a DMD patient presented a milder dystrophic clinical pathology, delayed loss of ambulation, and overall short stature due to a growth hormone (GH)-deficiency(78, 79). A double-blinded controlled study of monozygotic twin DMD boys in which one was administered the growth hormone inhibitor manzindol versus a

placebo revealed that the DMD twin receiving growth hormone inhibitor had greater mobility and reduced symptoms compared to his DMD twin on placebo(80). However, a larger double-blinded studies revealed that manzindol-treated DMD boys showed no significant benefit over placebo-treated individuals(81). DMD boys with growth hormone deficiency given growth hormone showed no acceleration of dystrophic disease progression in muscle or cardiopulmonary outputs(82, 83). It has been postulated based on natural history longitudinal studies in DMD boys and dystrophin-deficient animal models that short stature in DMD boys is beneficial for delaying the loss of ambulation(84). Mouse models of growth hormone-deficiency such as the *Ames/Dwarf* and Growth hormone receptor (*GHR*) mutant mice have revealed that short stature/growth retardation slows aging via alteration of metabolic responses to insulin/IGF1 signaling(85, 86). It is likely that the regulation of growth hormone may modulate DMD symptoms and influence phenotypic outcomes. The naturally occurring genetic variants resulting in short-stature/dwarfism in DMD patients have been reported to result in overall milder dystrophic pathology and better outcomes(87).

Due to the robust influence of inflammatory and fibrotic signaling in dystrophic muscle pathology, finding ways to alter these signaling pathways remains a promising strategy as more genetic modifiers have been identified. Recent whole exome analyses of the DMD population collected at various clinic sites have revealed additional genetic modifiers of DMD with ties to the TGFβ signaling pathway. Osteopontin is a secreted extracellular matrix protein thought to be essential for normal osteoclast formation and bone mineralization(88). Osteopontin protein was shown to be strongly increased in expression levels in the DMD patient muscle biopsies and the serum of *mdx* mice(89). The *OSTEOPONTIN* (*SPP1*) G allele polymorphism rs28357094 has been shown to be a predictive indicator of loss of ambulation and degree of muscle weakness in DMD patients(90, 91). Indeed, *Osteopontin* null mice show altered immune signaling and reduced muscle fibrosis in the *mdx* mice via reducing M1 and M2a macrophage populations to the more pro-regenerative M2c macrophage population subset(92). The *Latent TGFβ Binding Protein 4* (*LTBP4*) gene was initially identified from a screen of gamma-sarcoglycan (*Sgcg*) null mice for cytoprotective single nucleotide polymorphisms (SNPs) between two interbred mouse strains(93). Studies in DMD patients revealed that four *LTBP4* SNPs (V194I, T787A, T820A, and T1140M; VTTT/IAAM haplotype) were predictive of the age at onset of loss of ambulation and of dilated cardiomyopathy (DCM) onset(94, 95) (Table 1). Follow-up studies in mice and cell culture demonstrated that the IAAM residues bound more latent TGFβ compared to the LTBP4 VTTT protein(96). Thus, direct inhibition of LTBP4 via neutralizing antibodies or other RNA/protein antagonistic strategies may lead to a novel therapeutic means of blocking inflammation and myofiber breakdown in dystrophin-deficient skeletal muscles. Whole exome analyses of a large DMD cohort demonstrated that a SNP at rs1883832 of the *CD40* gene is predictive of the age at loss of ambulation and further implicated important roles in DMD for both the NFκB and TGFβ signaling pathways(97) (Table 1). CD40 is a cell surface receptor that is predominantly expressed on mature B cells as part of the Tumor Necrosis Factor (TNF) family of proteins(98). CD40 is required for immunoglobulin class switching and *CD40* human polymorphisms have also been linked to increased susceptibility to immunological diseases(99, 100). The immune system has long been shown to have an important role in normal muscle growth and

regeneration, as well as the progression of muscular dystrophies(101, 102). The *CD40* rs1883832 SNP is likely to have functional immunological consequences for the progression of DMD symptoms as the dystrophic muscle begins to deteriorate with the advancing of the disease.

Genetic modifiers of Facioscapulohumeral muscular dystrophy

Facioscapulohumeral muscular dystrophy affects 1:8000 individuals worldwide and is caused by a contraction of D4Z4 expansion repeats in the *DUX4* pseudogene resulting in the production of the myotoxic DUX4 protein(103, 104). DUX4 transcriptional activity is thought to activate pro-apoptotic signaling pathways, immune signaling regulators, and retrotransposons resulting in FSHD disease pathology(105). One of the more perplexing aspects of FSHD is the presence of the contracted D4Z4 repeat in asymptomatic familial carriers whom show no disease pathology(106, 107). It has been postulated that single nucleotide polymorphisms (SNPs) affecting DUX4 expression may allow for the permissive state of DUX4 transcriptional activation(108). Interestingly, mutations in the chromatin-modifying gene structural maintenance of chromosomes flexible hinge domain containing 1 (*SMCHD1*) result in the relaxing of chromatin thereby permitting the expression of the DUX4 protein and affected patients are categorized as FSHD type 2(109) (Figure 1). Additional FSHD epigenetic regulators including a long non-coding RNA (lncRNA) known as *DBE-T*, have been shown to regulate the expression of DUX4 in skeletal muscles via modulation of transcriptional regulatory complexes(110). The most interesting aspect of FSHD genetics is that many individuals in FSHD families have been identified as having the FSHD permissive allele, but do not have the phenotypic muscle weakness and thus cannot be diagnosed with FSHD(111). As more and more FSHD families and patients have their exomes and genomes fully-sequenced, additional genetic modifiers that may be consequential to disease progression and outcome will likely be identified.

Advances in DMD/FSHD therapeutic strategies and the potential use of corrective genome editing technologies

The identification of protective variants in human patients offers novel therapeutic entry points for the treatment of muscular dystrophies and muscle diseases in general. Improved adeno-associated viral (AAV) vectors for gene therapy and other biologics have been designed and delivered to patients to overexpress key cytoprotective muscle factors(112). As next-generation sequencing (NGS) has become cheaper and more accurate, larger-scale genomic analyses of healthy and diseased populations are now routinely being conducted. These studies are important to demonstrate the presence of genetic variations in specific regions of the human genome, and the function of transcriptional regulatory regions. The ENCODE (Encyclopedia of DNA Elements) project lists additional layers of epigenetic (non-DNA-dependent) regulation of gene expression of many common disease-causing genes(113). Non-coding RNAs (ncRNAs), RNA-splicing factors, and DNA methylation/acetylation factors among other epigenetic elements were shown to be important post-transcriptional regulators of human muscle diseases(114). The cost of whole genome

sequencing has dropped significantly, thus resulting in an increased consideration towards newborn screening of infants for diagnostic and epidemiological purposes(115, 116).

CRISPR genomic editing has emerged as a potential method for correcting small DNA mutations via targeting the specific mutation and replacing it with the corrected sequence(117). Several recent studies have shown that AAV-mediated CRISPR genomic editing can correct the dystrophin exon 23 mutation in the *mdx* mouse(118–121). Another recent study demonstrated up to 70% restoration of dystrophin protein expression in the myogenic area of AAV-CRISPR mediated correction of the mdx^{4cv} (exon 53 mutation) mouse(122). While these therapies may hold promise for direct correction of small *DMD* point mutations or deletions, the majority of DMD patients have large, multi-exon deletions for which CRISPR-mediated genomic corrective editing is not currently feasible(30). Nevertheless, it may be possible to alter the genomic sequences of patients with muscular dystrophies and insert or remove the functionality of a therapeutic genetic modifier via CRISPR technology.

Recently, the exon-skipping compound eteplirsen (previously referred to as AVI-4658, Sarepta Therapeutics) was conditionally approved by the US Food and Drug Administration (FDA) for the treatment of DMD patients with genetically amenable dystrophin mutations(123). Eteplirsen functions via bypassing the dystrophin mutation (skipping dystrophin exon 51) resulting in the production of a chimeric, partially-functional dystrophin protein that produces a Becker-like phenotype (124). In a clinical trial of DMD boys amenable to skipping dystrophin exon 51, it was shown that DMD boys treated with eteplirsen retained ambulation longer than natural history controls with the same mutations(125). Gene therapies that overexpress a truncated form of dystrophin (micro-dystrophin) have shown efficacy in DMD animal models and are in current clinical trials for DMD(126–128). The naturally-produced myokine myostatin (also called GDF8) has been shown to be a potent negative regulator of muscle mass in mammals(129). Subsequently, naturally occurring loss-of-function genetic mutations in the myostatin gene of Belgian Blue cattle was demonstrated to be the direct cause of the doubling of their muscle mass via muscle hypertrophy(130). Later, human case studies showed that myostatin genetic variants were responsible for the large, hypertrophic muscles in a young German boy(131). Genetic loss-of-function mutations of myostatin have been shown to induce muscle hypertrophy, and protect against muscle force deficits in *mdx* mice(132). Pharmacological blockade of myostatin or the myostatin receptor Activin IIB (ACVRIIB) similarly demonstrated a physiological benefit in protecting against muscle force loss in dystrophic mice(133, 134). A recent clinical trial involving a soluble form of the human activin receptor type IIB (ACE-031; produced by Acceleron Pharma) given to ambulatory DMD boys showed some benefits over placebo; although the trial was halted due to unforeseen side effects(135). A similar myostatin/TGF-β pathway inhibitory compound (ACE-081) is currently recruiting for a Phase 2 trial for FSHD patients to alleviate symptoms of muscle weakness (Clinical Trials Identifier: NCT02927080). While these compounds may ameliorate muscle weakness and other dystrophic symptoms, they do not correct the underlying cause of the muscle disease. Many additional strategies towards the treatment of DMD and FSHD are currently under development or in clinical trials and many of these therapeutic targets were originally identified from genomic modifiers of muscular dystrophies(136). It is likely that no single

treatment for DMD or FSHD will fully cure either disease, but combination treatments targeting multiple factors including those identified as genetic modifiers may improve muscle symptoms and extend the lifespans of affected individuals.

As genomic sequencing data from muscular dystrophies becomes more commonplace, the opportunity and ability to identify protective and pathogenic genetic modifiers of muscular dystrophies will increase dramatically. These novel genetic modifiers may hold biological clues as to why some individuals do not display muscular dystrophy symptoms despite having pathogenic variants. The exciting prospect of exploring these genetic modifiers as therapeutic agents for drug development may lead to novel treatments for treating these debilitating diseases.

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List of Abbreviations

AAV	Adeno-associated viral vector
ASD	Autism spectrum disorder
BMD	Becker muscular dystrophy
CNV	Copy number variant
CRISPR	Clustered regularly interspaced short palindromic repeats
DAPC	Dystrophin-associated protein complex
DCM	Dilated cardiomyopathy
DGC	Dystrophin-glycoprotein complex
DM	Myotonic dystrophy
DMD	Duchenne muscular dystrophy
DUX4	Double homeobox 4
ECM	Extracellular matrix
ENCODE	Encyclopedia of DNA Elements
ExAC	Exome Aggregation Consortium

FSHD	Facioscapulohumeral muscular dystrophy
GH/GHR	Growth hormone/Growth hormone receptor
GRMD	Golden Retriever muscular dystrophy
JAG1	Jagged-1
lncRNA	Long non-coding RNA
LoF	Loss-of-function
LTBP4	Latent TGFβ Binding Protein 4
mdx	X chromosome-linked muscular dystrophy
ncRNA	Non-coding RNAs
NGS	Next-generation sequencing
SMCHD1	Structural maintenance of chromosomes flexible hinge domain containing 1
SNP	Single nucleotide polymorphism
SPP1	Osteopontin
WES	Whole exome sequencing
WGS	Whole genome sequencing

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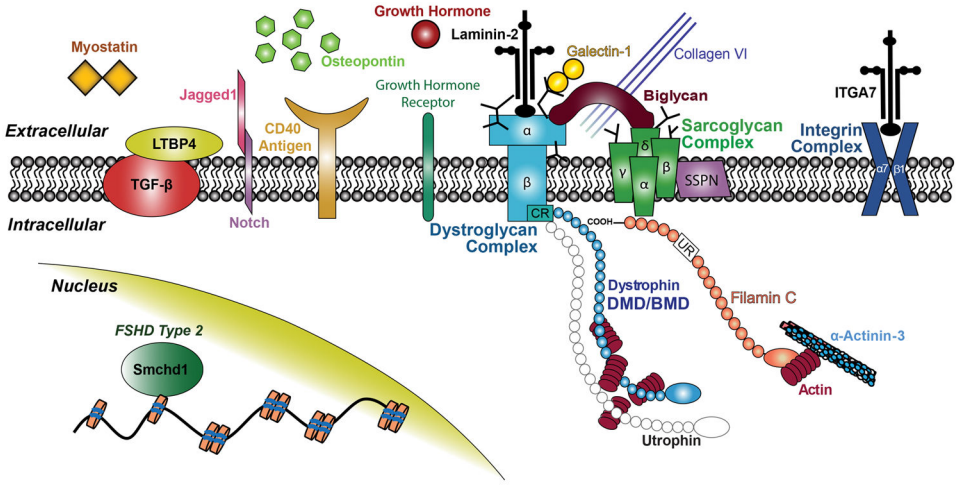

Figure 1. Schematic of significant genetic modifiers of Duchenne and Facioscapulohumeral muscular dystrophies and their sub-cellular localization in skeletal muscle

Dystrophin and the dystrophin-associated protein complex (DAPC) have an important functional role in the transmission of intercellular force to the extracellular matrix (ECM). Of note, Smchd1 is a chromatin-modifier protein that is believed to allow a permissive state for transcriptional activation of the pathogenic DUX4 transcription factor in FSHD Type 2.

Table 1

Genes with Polymorphic Significance in the Clinical Manifestation of Muscular Dystrophies

Gene Name	Abbreviation	Normal Function	Disease Modification	Modification-Associated Phenotype
Genes with Identified Mutation or Significant Variant Contributing to Pathological Variation				
α-Actinin-3	*ACTN3*	Skeletal muscle-specific actin binding protein	Homozygosity for SNP R577X in DMD patients	Reduced susceptibility to stretch-induced damage, enhanced fatigue recovery, and slowed dystrophic progression
Annexin A6	*ANXA6*	Calcium-dependent membrane and phospholipid binding protein	Exon 11 SNP rs26961431 creates a truncated Annexin A6 protein in mice	ANXA6 truncation results in increased muscle wasting and degeneration in *Sgcg* mutant mice
CD40 Antigen	*CD40*	Transmembrane receptor of the tumor necrosis factor receptor family; involved in T helper cell polarization	Minor allele rs1883832 in the 5′ UTR of CD40 associated with diminished CD40 expression	Differential factor for age of loss of ambulation in DMD patients
Growth Hormone/ Growth Hormone Receptor	*GH/GHR*	Regulates normal growth and IGF1 signaling in muscle	Growth hormone-deficiency comorbid with DMD in selected case reports	Case reports of DMD patients with dwarfism/short stature having delayed loss of ambulation
Latent TGFβ Binding Protein 4	*LTBP4*	Regulator of TGFβ and TGFβ signaling	1. IAAM haplotype in DMD patients	1. Reduced inflammation and myofiber breakdown, increased time to loss of ambulation
			2. Recessive T allele at rs10880 and IAAM haplotype in DMD patients	2. Protective against dilated cardiomyopathy (DCM)
Structural maintenance of chromosomes flexible hinge domain containing 1	*SMCHD1*	Chromatin modification; mediates CpG methylation and chromatin relaxation	In FSHD, allelic variants in SMCHD1 result in hypomethylation and inadvertent expression of DUX4; subsequent diagnosis of FSHD2, genotypically distinct from FSHD1.	FSHD type 2 allows for permissive expression of DUX4
Genes with Protein Expression Differences Contributing to Pathological Variation				
α-7 Integrin	*ITGA7*	Mediates cell-matrix interactions; receptor for basement membrane protein laminin-1	Increased expression in *mdx* mice via transgenic cassette or SU9516 compound	Reduced membrane injury, increased muscle fiber size, decreased levels of fibrosis, extended lifespan
Biglycan	*BGN*	Small leucine-rich repeat proteoglycan; ubiquitous structural ECM protein	Increased expression of Biglycan corresponds with stabilization of Utrophin	Decreased diseased muscle pathology
Galectin-1	*LGALS1*	Laminin-binding protein; mediates cell-matrix interactions	Increased expression in GRMD dogs	Enhanced muscle repair, reduced immune response and apoptosis
Jagged1	*JAG1*	Ligand of the Notch receptor	Increased expression in *mdx* mice	Improved dystrophic histology and physiology
Myostatin	*GDF8*	TGF-β superfamily member	Decreased expression in animals and humans	Doubling of muscle mass via myofiber hypertrophy
Osteopontin	*SPP1*	Diverse matricellular protein	1. Decreased expression in mice and humans	1. Decreased immune signaling and fibrosis, increased muscle regeneration
			2. Dominant G allele at SPP1 rs28357094	2. Protective against dilated cardiomyopathy (DCM)
Sarcospan	*SSPN*	A member of the DAPC, which provides a structural anchor of	Transgenic overexpression in *mdx*	Increased myofiber membrane stability and reduces muscle tearing

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		the myocyte cytoskeleton to the ECM	mice improves muscle function and force.	
Utrophin	*UTRN*	Autosomal homolog of DMD gene; maintains sarcolemmal integrity during embryonic myogenesis	Overexpression of Utrophin in mice or with Ezutromid compound	Increased lifespan, decreased diseased muscle pathology