

CHAPTER 32

Cardiomyopathy in muscular dystrophies

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Introduction

Muscular dystrophies encompass a group of distinct genetic disorders, sharing a common feature of skeletal muscle weakness and wasting that is believed to be due to a disease process that occurs within skeletal muscle cells. The pathological hallmarks of muscular dystrophy patients include high serum levels of cytosolic muscle enzymes and, upon muscle biopsy, evidence of ongoing muscle necrosis, muscle regeneration, interstitial fibrosis and fatty replacement. The vast genetic heterogeneity of the more than 30 forms of muscular dystrophy has been realized. It has long been recognized that many muscular dystrophy patients have significant risk of developing cardiovascular disease, with a description of a patient dying from an enlarged heart in one of the earliest known papers describing muscular dystrophy patients.¹ Given that there are significant similarities, both structural and functional, between cardiac and skeletal muscle, similar disease mechanisms may be involved in both skeletal muscle fibers and cardiac muscle cells. Elevations in cardiac-specific cytoplasmic markers such as cardiac troponin I in some muscular dystrophy patients may be analogous to release of creatine kinase from skeletal muscle. However, many proteins mutated in muscular dystrophy patients are also expressed in the vasculature or the cardiac conduction system, and have the potential to modify cardiovascular function in addition to

any primary defect in cardiac muscle cells themselves.

In this chapter, we summarize our current understanding of the genetic diversity and molecular mechanisms that lead to the development of cardiomyopathy in muscular dystrophy patients. Much of our current knowledge has been derived from smaller case studies, some natural history studies in human patients, and study of genetic mouse models. Together, these studies are beginning to form a framework for important recommendations regarding clinical care of human patients. Hopefully, with additional basic research, and much needed multicenter clinical assessments and trials, pharmacological and/or genetic therapies can be rigorously tested and applied to prevent the important clinical problem of heart disease in the muscular dystrophy patient population. More broadly, the work on genetically defined forms of cardiomyopathy will hopefully provide important insight into the critical molecular mechanisms behind the more common acquired forms of heart disease.

Overview

The proteins affected by genetic mutations that cause muscular dystrophy are localized to a number of key functional systems within muscle cells, including the extracellular matrix, the muscle cell

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membrane, the intracellular cytoskeleton, the secretory pathway, the sarcomere and even the nuclear envelope (Fig. 32.1). Although, in general, cardiomyopathy is a recognized clinical problem in many forms of muscular dystrophy, in some cases it appears that cardiac muscle is spared (Table 32.1). This may be due to differential expression of the

mutated gene in skeletal muscle, compared to cardiac muscle and smooth muscle, but also may be due to differences in the significance of the functional processes affected by the mutation in cardiac or skeletal muscle. There is not an extensive amount of large-scale natural history data determining the relative risk of cardiomyopathy in

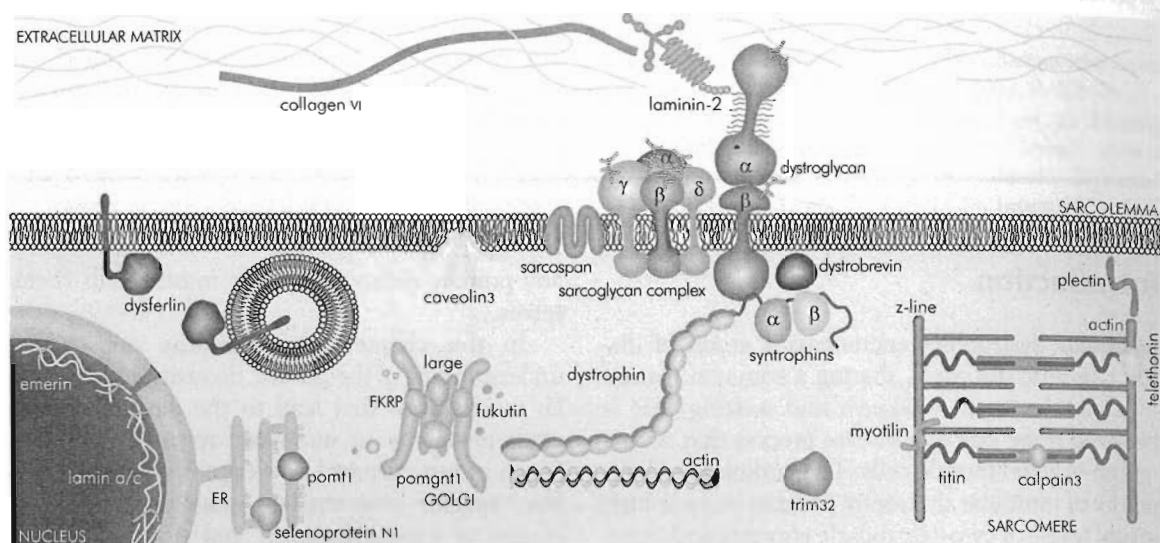


Figure 32.1. Muscle proteins and molecular pathways involved in the pathogenesis of human muscular dystrophies.

Table 32.1. Cardiac involvement in human muscular dystrophies.

Gene mutated	Muscular dystrophy	Inheritance	Cardiac involvement
Membrane-associated proteins			
dystrophin (<i>DMD</i>)	Duchenne muscular dystrophy	X-linked	Very common
	Becker muscular dystrophy	X-linked	Very common
α -sarcoglycan (<i>SGCA</i>)	Limb girdle muscular dystrophy 2D	AR	Rare
β -sarcoglycan (<i>SGCB</i>)	Limb girdle muscular dystrophy 2E	AR	Common
γ -sarcoglycan (<i>SGCG</i>)	Limb girdle muscular dystrophy 2C	AR	Common
δ -sarcoglycan (<i>SGCD</i>)	Limb girdle muscular dystrophy 2F	AR	Common
caveolin 3 (<i>CAV3</i>)	Limb girdle muscular dystrophy 1C	AD	None reported
	Rippling muscle disease	AD	Rare
	HyperCKemia	AD	None reported
dysferlin (<i>DYSF</i>)	Limb girdle muscular dystrophy 2B	AR	Rare
	Miyoshi myopathy	AR	None reported
plectin (<i>PLEC</i>)	Epidermolysis bullosa with muscular dystrophy	AR	None reported

Table 32.1. Cardiac involvement in human muscular dystrophies – continued

Gene mutated	Muscular dystrophy	Inheritance	Cardiac involvement
Enzymes involved in glycosylation			
fukutin (<i>FCMD</i>)	Fukuyama congenital muscular dystrophy	AR	Common
fukutin-related protein (<i>FKRP</i>)	Limb girdle muscular dystrophy 2I	AR	Common
	Congenital muscular dystrophy 1C	AR	Common
protein O-mannosyltransferase 1 (<i>POMT1</i>)	Walker–Warburg syndrome	AR	None reported
protein O-mannosyl- <i>N</i> -acetylglucosaminyl transferase (<i>POMGNT1</i>)	muscle-eye-brain disease	AR	None reported
<i>LARGE</i>	Congenital muscular dystrophy 1D	AR	None reported
Extracellular matrix proteins			
laminin α_2 (merosin) (<i>LAMA2</i>)	Merosin-deficient congenital muscular dystrophy	AR	Rare
collagen VI (<i>COL6</i>)	Ullrich congenital muscular dystrophy	AR	None reported
	Bethlem myopathy	AD	Rare
Sarcomeric proteins			
Titin (<i>TTN</i>)	Tibial muscular dystrophy/LGMD2J	AR	None reported*
calpain-3 (<i>CAPN3</i>)	Limb girdle muscular dystrophy 2A	AR	None reported
telethonin (<i>TCAP</i>)	Limb girdle muscular dystrophy 2G	AR	Common
myotilin (<i>TTID</i>)	Limb girdle muscular dystrophy 1A	AD	None reported
Nuclear membrane proteins			
emerin (<i>EMD</i>)	Emery–Dreifuss muscular dystrophy	X-linked	Very common
lamin A/C (<i>LMNA</i>)	Emery–Dreifuss muscular dystrophy	AD	Very common
	limb girdle muscular dystrophy 1B	AD	Very common
Cytosolic proteins			
<i>TRIM32</i>	Limb girdle muscular dystrophy 2H	AR	None reported
selenoprotein N1 (<i>SEPN1</i>)	Rigid spine congenital muscular dystrophy	AR	Common?
Nucleotide repeats			
<i>D4Z4</i> region of chromosome 4	Facioscapulohumeral muscular dystrophy	AD	Rare
<i>DMPK</i> (CUG repeat)	Myotonic dystrophy 1	AD	Very common
<i>ZNF9</i> (CCUG repeat)	Myotonic dystrophy 2	AD	Very common
<i>PABPN1</i> (poly-alanine)	Oculopharyngeal muscular dystrophy	AD/AR	None reported

Muscular dystrophies are classified by the proposed function of the gene (mRNA). Muscular dystrophy nomenclature is by the convention of the World Muscle Society and cardiac involvement is indicated. *Mutations in the *TTN* can cause DCM, but mutations in *TTN* causing muscular dystrophy do not seem to cause heart disease. AD, autosomal dominant; AR, autosomal recessive.

muscular dystrophies, but small clinical reports and anecdotal evidence indicate that in many cases, the risk of cardiovascular disease is probably very significant. There are a number of excellent recent reviews that describe the cardiovascular complications of some of the muscular dystrophies in greater detail.²⁻⁸ In some forms of muscular dystrophy, the European Neuromuscular Centre has proposed some guidelines for clinical cardiovascular care.⁹ In addition, research in mouse models of muscular dystrophies has strongly supported the possibility that pharmacological or genetic therapies will be able to correct or improve cardiovascular disease in muscular dystrophy patients.

Because of the vast genetic heterogeneity of genes causing muscular dystrophy, and because current classification systems of muscular dystrophies are based in part on the historical order of clinical and genetic identification, we will instead focus on the functional compartments of the muscle cell affected by these mutations (Table 32.1).

Membrane-associated proteins

Mutations in several proteins localized to the plasma membrane of muscle cells have been implicated in the pathogenesis of various forms of muscular dystrophy. The proteins of the dystrophin-glycoprotein complex (Fig. 32.1) appear to play a critical role in linking the sub-membrane cytoskeleton to the extracellular matrix. In addition, several other molecules that regulate membrane structure or membrane-bound signaling proteins appear to be important in human muscular dystrophies. Many of these muscular dystrophies associated with defects in the muscle cell plasma membrane present with clinically significant cardiovascular disease.

Dystrophinopathies

The first mutation identified to cause muscular dystrophy was in a protein called dystrophin which causes two X-linked forms, Duchenne's (DMD) and Becker's muscular dystrophy (BMD).^{10,11} The dystrophin gene is one of the largest genes in nature with 79 exons, and alterations in promoter

usage and splicing can generate several dystrophin isoforms.¹² The major form expressed in skeletal and cardiac muscle is the full-length 427 kDa isoform. Dystrophin is anchored to the sarcolemma and the muscle cell plasma membrane, through its association with the transmembrane dystrophin-glycoprotein complex (DGC), which includes dystrophin, dystroglycan, sarcoglycans, sarcospan, syntrophins and dystrobrevin.¹³ The N terminus of dystrophin binds to the actin cytoskeleton, a long rod domain containing 24 spectrin-like repeats, and regions near the C terminus bind to dystroglycan (Fig. 32.1). Therefore, dystrophin, by anchoring the DGC to the actin cytoskeleton, is thought to provide structural support to the muscle cell membrane and prevent membrane damage during cycles of contraction and relaxation.¹⁴ Mutations in dystrophin cause loss of dystrophin from the muscle cell membrane and a concomitant reduction in the expression of the DGC.^{15,16} Although dystrophin is also expressed in smooth muscle, there is also significant expression of a dystrophin homolog called utrophin in smooth muscle, and utrophin appears to be able to maintain DGC expression in smooth muscle in the absence of dystrophin.¹⁷

DMD was first identified in the mid 1800s, and predominantly affects boys (X-linked) with a frequency of 1 in 3500 male births, with clinical symptoms usually appearing between the ages of 3 and 6.¹⁸ By the early teens, DMD patients are often wheelchair bound, require respiratory therapy, and nearly always die in their late teens or early twenties. Although the exact percentages are uncertain, it has been estimated that roughly 15-20% of DMD patients die from heart failure or cardiomyopathy, and the rest from predominantly respiratory failure (40%) or a combination of pneumonia, respiratory failure and cardiovascular complications.^{4,19,20} At least 90% of older DMD patients have some form of cardiovascular involvement detectable by either electrocardiogram (ECG) or echocardiography.⁴ Sinus tachycardia is almost always present after age 5, and is usually the first sign of cardiac involvement.^{4,21} Many patients progress to dilated cardiomyopathy, although sometimes hypertrophy is seen early on.²² In contrast, BMD patients only develop mild skeletal

muscle disease, often do not require a wheelchair, and can live a near normal lifespan. However, despite their mild skeletal muscle disease, heart muscle can be severely affected, with cardiac death due to heart failure occurring in up to 50% of patients.⁴ Many BMD patients can present with cardiomyopathy, while having little or no symptoms of skeletal muscle weakness.²³ Cardiovascular involvement occurs in up to 90% of BMD patients, detectable by echocardiography and ECG, with disease often progressing to dilated cardiomyopathy or severe heart failure requiring cardiac transplant.^{4,24,25} It is not clear if the high prevalence of cardiomyopathy with mild skeletal muscle disease in BMD patients is solely due to a longer lifespan in which cardiac disease can become apparent, or whether somehow the specific mutations that cause mild BMD differentially severely affect cardiac muscle and while only having mild effects on skeletal muscle.

The importance of dystrophin in the development of heart disease is also supported by the identification of mutations in dystrophin that cause X-linked dilated cardiomyopathy (XLDCM).^{26,27} XLDCM is often described as an exclusively cardiovascular disease, a particularly rapidly progressing heart disease that usually affects males in their teenage years. In many cases of XLDCM, patients do have mild skeletal muscle involvement which blurs the distinction between XLDCM and BMD.^{4,28} However, certain XLDCM causing mutations in the 5' end of the dystrophin gene appear to regulate the interaction of the dystrophin promoter with various molecules that regulate gene transcription.^{27,29} In many of these patients, dystrophin is completely absent in cardiac muscle, but skeletal muscle shows upregulation of non-muscle dystrophin isoforms.^{27,29} Therefore, differences in dystrophin gene expression regulation in cardiac and skeletal muscle may explain the cardiovascular-specific features of certain XLDCM patients.

Female carriers of BMD and DMD mutations would also be predicted to have partial dystrophin deficiency in the myocardium, due to random X-inactivation in cardiac myocytes. Indeed, up to 90% of adult female DMD carriers have significant cardiovascular involvement with echocardiographic and ECG changes, and up to 10% develop

cardiac failure.^{4,30} Because these patients are generally asymptomatic with respect to both cardiac and skeletal muscle disease, this group should be monitored closely for signs of preclinical cardiovascular disease.

The major mechanism for dystrophin-related cardiomyopathy is probably due to disruption of DGC function, leading to destabilization of the cardiac myocyte membrane, and resulting in increased myocardial cell necrosis. Unlike skeletal muscle, cardiac muscle is unable to undergo significant amounts of regeneration, and necrotic tissue is replaced with connective tissue and/or fat. More significant ECG changes appear to reflect scarring of the left ventricular wall. The fibrotic changes in the heart are probably responsible for the changes in electrical conduction seen in human patient ECGs,^{31,32} The left ventricle appears more significantly affected in DMD patients, perhaps indicating that increased myocardial wall stress induces contraction-induced damage.³³ The compensation of dystrophin deficiency by utrophin appears to maintain the utrophin-glycoprotein complex expression in smooth muscle.¹⁷ However, there is some evidence in human patients that blood flow, at least to the limb muscles, is altered in DMD patients.³⁴ Data from mouse models suggest the mechanism of alterations in blood flow might be due to abnormal amounts of nitric oxide produced by neuronal nitric oxide synthase (NOS) in skeletal muscle.^{35,36} Neuronal NOS is associated with the DGC in striated muscle cells, and regional nitric oxide normally counterbalances α -adrenergic-stimulated contraction of the microvasculature during the 'fight or flight' response. It is unclear whether this mechanism is involved in cardiomyopathy, as positron emission tomography studies have not consistently shown abnormal or reduced coronary blood flow in human DMD and BMD patients.³⁷

Mdx mice have naturally occurring mutations in the dystrophin gene, and display many features of human muscular dystrophy.³⁸ There is pathological evidence of cardiomyopathy, and histological evidence of necrosis in the hearts of mdx mice beginning around 24 months of age, along with elevated levels of cardiac troponin I.³⁹ The cardiovascular phenotype in mdx mice appears to worsen

under conditions of pressure overload, or isoproterenol infusion, suggesting cardiovascular stress plays a significant modulatory role in disease pathogenesis.^{40,41} Mdx/myoD double knockout mice have a more severe cardiomyopathy, even though myoD is expressed in skeletal muscle cells, suggesting that a more severe muscular dystrophy might exacerbate the cardiovascular phenotype.⁴² Transgenic expression of the dystrophin gene specifically within cardiac myocytes specifically rescues the cardiovascular phenotype within mdx mice.⁴³ Mdx mice do not show evidence of coronary vasospasm, nor do they respond to agents which prevent coronary vasospasm in other mouse models. This suggests that the primary defect in mdx mice causing cardiomyopathy is a defect within the cardiac myocyte, and not secondary to a deficiency of dystrophin within smooth muscle of the coronary vasculature.³⁹

Sarcoglycanopathies

Sarcoglycans were originally identified by biochemical purification of the DGC in skeletal muscle.¹³ There are six known sarcoglycan genes in humans, α , β , γ , δ , ϵ and ζ . The sarcoglycans are believed to assemble as a five-protein complex of four single pass transmembrane sarcoglycans, and a tetraspan-like protein sarcospan (Fig. 32.1).⁴⁴ The major striated muscle sarcoglycan complex in the DGC is composed of α -, β -, γ - and δ -sarcoglycan.¹³ ϵ -sarcoglycan shares significant sequence identity with α -sarcoglycan, but is expressed much more broadly in nearly all tissues.⁴⁵ ϵ -sarcoglycan replaces α -sarcoglycan, and assembles with β -, γ - and δ -sarcoglycan in the smooth muscle sarcoglycan-sarcospan complex.⁴⁶ ζ -sarcoglycan was recently described and appears to be expressed in skeletal muscle and smooth muscle, but it is still unclear whether it is an essential component of the major sarcoglycan complexes in these tissues.⁴⁷

Subsequent to the identification of sarcoglycans in the DGC, mutations in α -, β -, γ - and δ -sarcoglycan were identified in limb girdle muscular dystrophies 2C-2F, respectively.⁴⁸⁻⁵³ Patients generally develop a muscular dystrophy that is variable between DMD-like and BMD-like severity, and cardiomyopathy is common in patients with mutations in β -, γ - and δ -sarcoglycan mutations. Signs

of cardiac involvement (ECG or echocardiographic abnormalities) have been reported in approximately 30-70% of sarcoglycanopathy patients, with nearly 20% showing clear signs of dilated cardiomyopathy.⁵⁴⁻⁵⁶ Interestingly, mutations in ϵ -sarcoglycan in humans do not cause muscular dystrophy, but instead are responsible for a hereditary form of myoclonus dystonia.⁵⁷ Finally, heterozygous mutations in δ -sarcoglycan have been identified in patients with inherited and idiopathic dilated cardiomyopathy (DCM).¹³ Interestingly, these patients do not appear to have any skeletal muscle disease. It is unclear what accounts for the apparent tissue-specific effect of these particular DCM mutations in δ -sarcoglycan.

Interest in the smooth muscle expression of the sarcoglycan complex originated largely from mouse studies where the sarcoglycan genes have been knocked out. In particular, α -sarcoglycan knockout mice do not develop significant evidence of cardiomyopathy, while β - and δ -sarcoglycan knockout mice develop cardiomyopathy.⁵⁸⁻⁶¹ Interestingly, while all three lines have the deficiencies of the sarcoglycan complex in cardiac muscle, the latter two lines of mice have the sarcoglycan complex disrupted in smooth muscle in addition to cardiac muscle (Fig. 32.2).^{58,59} Morphological studies showed evidence of coronary vasospasm in these mice, as well as vasospasm in skeletal muscle and other non-muscle tissues.⁵⁹ Furthermore, the cardiomyopathy could be prevented in β - and δ -sarcoglycan knockout mice by pharmacological agents, nicorandil or verapamil, which prevented vasospasm (these agents could not prevent mdx mouse cardiomyopathy).^{39,58} Together these results have suggested that the disruption of the sarcoglycan complex causes a primary defect in smooth muscle function that contributes to the pathogenesis of cardiomyopathy. Similar results of coronary vasospasm have been reported in γ -sarcoglycan knockout mice, which also develop cardiomyopathy.⁶² There is some disagreement in the field over the expression distribution of γ -sarcoglycan in animal models, but it appears clear that in human patients with mutations in γ -sarcoglycan the entire smooth muscle sarcoglycan complex is disrupted.¹⁷ Transgenic expression of γ -sarcoglycan or δ -sarcoglycan

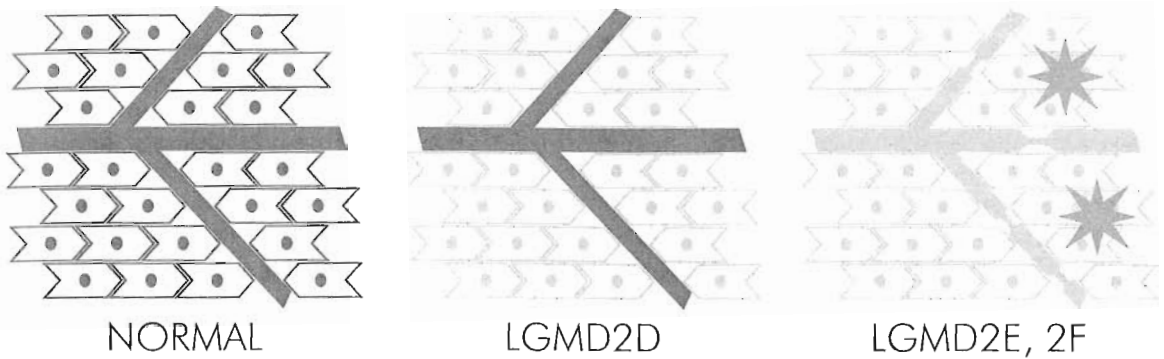


Figure 32.2. Cardiovascular distribution of the sarcoglycan complex in cardiac myocytes and blood vessels contributes to the pathogenesis of vasospasm and cardiomyopathy. In α -sarcoglycan-deficient patients (LGMD2D) the cardiac myocyte sarcoglycan complex is disrupted, but the sarcoglycan complex expression in smooth muscle is maintained by expression of ϵ -sarcoglycan. In β - and δ -sarcoglycan-deficient patients (LGMD2E and 2F), the smooth muscle sarcoglycan complex is also disrupted and vasospasm and cardiomyopathy occurs.

specifically in cardiac myocytes of corresponding knockout mice can prevent cardiomyopathy, and suggests that the smooth muscle disruption of the sarcoglycan complex alone is not sufficient to cause cardiomyopathy.⁶³ These data together support the hypothesis that the disruption of the sarcoglycan complex in cardiac myocytes is critical to rendering the muscle cells more susceptible to ischemic challenges resulting from the coronary vessel dysfunction.⁶⁴

Defects in the sarcoglycan complex are also responsible for inherited cardiomyopathy in inbred hamster lines.^{65,66} The cardiomyopathic hamster displays evidence of coronary vasospasm that is responsive to verapamil, and is thought to play a significant role in the focal necrosis seen in the heart.⁶⁷ Interestingly, two sublines, Bio14.6 and T0-2, were inbred specifically for hypertrophic cardiomyopathy and dilated cardiomyopathy, respectively. However, both lines have the same deletion of the first exon of the gene encoding δ -sarcoglycan.⁶⁸ It is still unclear how the same mutations in δ -sarcoglycan result in different forms of cardiomyopathy. Clearly, genetic modifiers between the two hamster strains probably play an important role in the differing pathogenesis of these two strains of animals.

Caveolinopathies

Caveolins comprise a three-member gene family that encode essential components of plasma membrane invaginations called caveolae.⁶⁹ Caveolae are specializations of the plasma membrane and are rich in proteins involved in cellular signaling. Caveolin-3 is a muscle-specific caveolin isoform. Caveolin-3 is expressed in skeletal, cardiac and smooth muscle, and is localized to the plasma membrane in striated muscle.⁷⁰ However, in smooth muscle, caveolin-1 is the predominant isoform localized to the muscle cell membrane.⁷¹

Dominant mutations in caveolin-3 have been identified in four different forms of muscle disease including limb girdle muscular dystrophy 1C (LGMD1C), rippling muscle disease (RMD), distal myopathy and hyperCKemia.⁷²⁻⁷⁶ The clinical features of each disease are distinct with, for example, LGMD1C having a typical muscular dystrophy phenotype and RMD having distinctive percussion-induced muscle contractions. Although each disease has differences in clinical presentation, there can clearly be overlap, with the same mutations causing several different disorders, occasionally even within the same family.^{77,78} There is one report of patients with homozygous mutations in caveolin-3, and they appear to have a more severe phenotype.⁷⁹

Cardiomyopathy has not been reported in LGMD1C. In fact, some mutations have been shown to cause severely decreased skeletal muscle caveolin-3 membrane localization, while having little effect on cardiac muscle caveolin-3 localization.⁸⁰ There have been two cases of RMD where patients were concluded to have died from cardiac arrhythmias but were later shown to have CAV3 mutations,⁸¹ and there is a form of recessive RMD where severe cardiac arrhythmias are common.⁸² Despite, the relative lack of cardiomyopathy in muscular dystrophy patients, mutations in caveolin-3 have been recently described in a family with hypertrophic cardiomyopathy, by a candidate gene approach.⁸³ The patients in this family did not have muscular dystrophy. Interestingly the identified mutated residue is also a target for an LGMD1C mutation, albeit a different substitution. The molecular basis for the apparent tissue specificity of these mutations at the same codon of caveolin-3 is unclear.

Despite the lack of evidence for cardiomyopathy being common in caveolin-related muscular dystrophy patients, mouse models of caveolinopathies have suggested that caveolin-3 may indeed be important for cardiovascular function, at least in the mouse. The caveolin-3 knockout mouse displays evidence for a mild muscular dystrophy and progressive cardiomyopathy with hypertrophy, dilation and fibrosis.⁸⁴ In addition, transgenic overexpression of caveolin-3 in the heart also produces a similar cardiomyopathic phenotype.⁸⁵ Finally, transgenic overexpression of a LGMD1C mutant caveolin-3 in skeletal muscle and in heart causes muscular dystrophy and hypertrophic cardiomyopathy.⁸⁶ Therefore, the mouse models that increase or eliminate caveolin-3 expression, or increase expression of mutant caveolin-3 expression, have not clarified exactly how dominant mutations in caveolin-3, that generally result in protein mislocalization, cause disease in human patients. However, several mechanisms including alterations in mitogen-activated protein kinase (MAPK) signaling, NOS signaling, or alterations of interactions with either the DGC or dysferlin have been proposed.⁷⁷

Dysferlinopathies

Dysferlin is a transmembrane protein that is expressed in both the sarcolemma and intracellular

vesicles.⁸⁷ Mutations have been linked to two allelic forms of muscular dystrophy that primarily affect the distal or the limb girdle muscles, Miyoshi myopathy and LGMD2B, respectively.⁸⁸ There have been no reports of cardiomyopathy in Miyoshi myopathy, but there are a few individual LGMD2B patients presenting with ECG abnormalities or Holter ECG abnormalities.⁸⁹ Recently, a mouse model of complete dysferlin deficiency showed dysferlin is involved in the muscle membrane repair process by regulating the Ca²⁺-dependent fusion of vesicles to repair holes in the plasma membrane.⁹⁰ Interestingly, dysferlin localization is altered in many forms of muscular dystrophy, suggesting that this membrane repair pathway may be activated, and dysferlin may play a modulatory role in other forms of muscular dystrophy.^{87,91} Therefore, the potential role of dysferlin in membrane repair in the heart awaits further examination. The SJL inbred mouse strain has a naturally occurring mutation in dysferlin that decreases dysferlin protein expression.⁹² In addition, there is a dysferlin homolog called myoferlin, which is expressed in skeletal muscle, and the heart and may compensate for the loss of dysferlin in certain striated muscles.⁹³

Epidermolysis bullosa with muscular dystrophy (plectin)

Plectin is localized to the Z-discs in striated muscle and to places where the cytoskeleton interacts with the membrane such as intercalated discs, desmosomes and gap junctions in the heart.⁹⁴ Mutations in plectin have been linked to autosomal recessive epidermolysis bullosa with muscular dystrophy.^{95,96} Patients with plectin mutations have severe skin blistering and a late-onset mild muscular dystrophy. No cardiovascular involvement has been reported. Plectin-null mice die at 2–3 days of age, and have skin blistering, mild skeletal muscle necrosis, and disruptions of the intercalated discs in the heart.⁹⁷

Rigid spine congenital muscular dystrophy (selenoprotein N1)

Selenoproteins are enzymes involved in oxidation–reduction reactions and have a selenocysteine residue in the catalytic site. Selenoprotein N1 (SEPN1) is localized to the endoplasmic reticulum

and appears to be an integral membrane protein.⁹⁸ Mutations in *SEPN1* have been linked to rigid spine congenital muscular dystrophy and a myopathy called multimincore disease.^{99,100} There are several early reports of patients with a rigid spine syndrome and cardiac involvement, but it is not completely clear whether any or all of these patients have *SEPN1* mutations.^{101–104} Selenium deficiency in the diet in humans is associated with a cardiomyopathy called Keshan disease.¹⁰⁵ Keshan disease is an interesting disorder of endemic cardiomyopathy resulting from outbreaks of myocarditis in children in rural China. Fortunately, Keshan disease can be completely prevented by oral sodium selenite.¹⁰⁶ Keshan disease appears to be caused by the selenium deficiency resulting in both an increased susceptibility to Coxsackievirus-induced myocarditis, and alterations in virulence of certain coxsackievirus strains.^{107,108} Related to muscular dystrophy-associated cardiomyopathy, coxsackievirus protease 2A has been shown to cleave dystrophin in experimental myocarditis (described later in this chapter, page 555).

Enzymes involved in glycosylation

Despite the importance of the DGC in muscular dystrophy and associated cardiomyopathy described above, there have been no mutations in humans described in the central protein of this complex, dystroglycan. Dystroglycan plays a critical role in the DGC as it binds intracellularly to dystrophin and extracellularly to laminin and other extracellular matrix proteins, thereby providing a critical link between the cytoskeleton and the extracellular matrix (Fig. 32.1).¹⁰⁹ Recently, a number of muscular dystrophies have been characterized with mutations in proteins that appear to be involved in glycosylation of glycoproteins.^{110,111} These enzymes are critical in the glycosylation pathway of dystroglycan, and the abnormal glycosylation of dystroglycan in these patients disrupts its function as an extracellular matrix receptor.¹¹² Therefore, these disorders can be thought of as 'dystroglycanopathies' because of the disruption of the functional link from the cytoskeleton to the extracellular matrix through dystroglycan. Similar

to mutations in other components of the DGC, cardiomyopathy seems to be an important feature in 'dystroglycanopathy' patients. In addition, many of these patients have abnormal central nervous system development and eye development, and severe mental retardation. The traditional knockout of the dystroglycan gene in the mouse is embryonic lethal due to a malformation of embryonic basement membranes.¹¹³ However, the specific deletion of the dystroglycan gene in skeletal muscle causes muscular dystrophy.^{114,115} The loss of dystroglycan function in the central nervous system in the mouse recapitulates nearly all of the neurological features of these disorders.¹¹⁶

Fukuyama congenital muscular dystrophy

Fukuyama congenital muscular dystrophy (FCMD) primarily affects the Japanese population, is the second most common muscular dystrophy in Japan (to Duchenne muscular dystrophy), and the majority of cases are linked to a founder mutation in the gene encoding fukutin (FCMD).¹¹⁷ FCMD has sequence similarity to the Fringe family of glycosyltransferases and is localized to the Golgi apparatus, but its exact function is unknown.^{118,119} FCMD patients have been shown to be deficient in normally glycosylated α -dystroglycan and α -dystroglycan-dependent ligand-binding activity in FCMD skeletal muscle is disrupted.^{112,120} There are several reports of severe myocardial fibrosis in autopsied specimens of FCMD cases.^{19,121} In a post-mortem study of 10 FCMD patients, one died of confirmed heart failure, and eight died of sudden death of undetermined causes.¹²² In addition, the fully glycosylated form of α -dystroglycan is not detectable in heart samples from FCMD patients.¹²⁰ A chimeric mouse model from fukutin-deficient embryonic stem cells has been produced (the knockout mouse is embryonic lethal) with brain and muscle phenotypes similar to human patients, but no cardiac characterization was reported.¹²³

Limb girdle muscular dystrophy 2I and MDC1C (fukutin-related protein)

Mutations in a protein with sequence homology to fukutin, fukutin-related protein (FKRP), have

been described in patients with limb girdle muscular dystrophy (LGMD2I) and congenital muscular dystrophy (MDC1C).^{124,125} There is a reduction in the fully glycosylated form of α -dystroglycan in skeletal muscle of patients with FKR mutations.¹²⁶ The exact function of FKR is unknown, but FKR is localized to the Golgi body.¹²³ Originally, mutations in FKR were thought to spare the brain, but recent evidence indicates that rare patients can have developmental abnormalities similar to FCMD and muscle-eye-brain disease.¹²⁷ LGMD2I is milder and of later onset than MDC1C, and all LGMD2I patients share a common founder missense mutation (L276I).¹²⁸ The carrier frequency of the missense mutation is as high as 1:400 in the United Kingdom. In general the severity of LGMD2I is milder than Duchenne muscular dystrophy. Similar to Duchenne muscular dystrophy and sarcoglycanopathies, dilated cardiomyopathy is a frequent complication in LGMD2I patients (~45% of patients), and left ventricular functional defects are common in MDC1C even before age 10.^{128,129}

Walker-Warburg syndrome and muscle-eye-brain disease (POMT1 and POMGNT1)

Walker-Warburg syndrome (WWS) and muscle-eye-brain disease (MEB) were originally thought to be allelic disorders due to the similarities of their clinical presentation including congenital muscular dystrophy, abnormal brain and eye development, and severe mental retardation.^{130,131} The clinical features of WWS and MEB are similar to severe FCMD, and patients with severe FKR mutations. WWS is generally more severe than MEB, with patients failing to survive past three or four years of age. Mutations in WWS and MEB patients have been described in genes encoding enzymes involved in O-mannosylation within the secretory pathway, in genes encoding an O-mannosyltransferase (POMT1) and the O-mannosyl-N-acetyl-glucosamine transferase (POMGNT1), respectively.^{132,133} Dystroglycan contains relatively unique O-mannosyl linked sugars that are believed to be important for ligand binding.¹³⁴ WWS and MEB patient skeletal muscle shows abnormal glycosylation of α -dystroglycan, and a loss of dystro-

glycan matrix receptor function.^{112,135} Because there are no additional homologs for POMGNT1 in humans, and POMT2 is required for POMT1 function, it is likely these mutations also effect glycosylation of dystroglycan in the heart. No cardiac phenotypes have been reported in WWS or MEB. However, due to the rarity and severity of these disorders, and the young age of the patients at the time of diagnosis, it could be that cardiovascular phenotypes have not yet developed or are overlooked.

Congenital muscular dystrophy 1D (LARGE)

LARGE is a protein with two domains that have homology to glycosyltransferases. An association of LARGE in muscular dystrophy was first shown by identification of mutations in LARGE through genetic linkage in the spontaneous mutant myodystrophy mouse (*myd*).¹³⁶ *myd* mice have abnormal glycosylation of dystroglycan similar to FCMD and MEB in both muscle and brain resulting in a loss of ligand-binding activity.¹¹² *myd* mice also have brain abnormalities identical to those of the mice with the dystroglycan gene (*DAG*) knocked out in the brain, and recapitulate features of human lissencephaly.^{112,116} Mutations in LARGE have been recently reported in a single patient with muscular dystrophy and severe mental retardation, but no cardiovascular phenotype was reported.¹³⁷ However, evidence of late onset focal myocardial necrosis/fibrosis has been shown in *myd* mice suggesting cardiac involvement might be seen in human patients.¹³⁸

Extracellular matrix proteins

Merosin (laminin-2)-deficient muscular dystrophy

Laminin is a heterotrimer of α , β and γ subunits, and resides in the extracellular matrix.¹³⁹ Laminins can bind to dystroglycan through their C-terminal G-domains, thereby completing the link from the actin cytoskeleton through dystrophin and dystroglycan to the extracellular matrix. Mutations in the laminin α_2 chain (merosin) cause merosin-deficient congenital muscular dystrophy.¹⁴⁰ Laminin α_2

is expressed in the heart, but there is only one small report where one out of six patients had significant cardiac involvement with a reported congestive cardiomyopathy at 1.4 years of age.¹⁴¹ The *dy/dy* mouse is a naturally occurring model of laminin α_2 -deficient muscular dystrophy, and has been reported preliminarily to have some cardiac involvement, although the lifespan of this mouse is only around 8 weeks.¹⁴² The lack of significant cardiac involvement in most patients could be due to compensation by other laminin α chains in the heart, or perhaps due to the young age at the time of diagnosis.

Bethlem myopathy and Ullrich congenital muscular dystrophy (collagen VI)

Collagen VI is a component of collagen fibrils in the extracellular matrix. Increased collagen deposition is often a consequence of myocardial remodeling following necrosis. Dominant mutations in the gene encoding collagen VI (*COL6*) cause Bethlem myopathy, and recessive mutations in *COL6* have been linked to Ullrich congenital muscular dystrophy.^{143,144} Cardiac involvement with hypertrophic cardiomyopathy has been reported in single patients with Bethlem myopathy (one out of 27 examined), but overall cardiac involvement appears to be rare in these muscular dystrophies.¹⁴⁵ A mouse model of collagen VI deficiency has been recently characterized with a mild myopathy, but no cardiovascular phenotype was reported.¹⁴⁶

Sarcomeric proteins

The sarcomere is the key functional unit within muscle necessary to produce both force and motion. It is well known that mutations in sarcomere proteins can affect heart function. Mutations in sarcomere proteins are responsible for nearly all cases of familial hypertrophic cardiomyopathy, and mutations in sarcomere proteins have been identified in dilated cardiomyopathy patients.¹⁴⁷ Many hypertrophic cardiomyopathy patients surprisingly do not have skeletal muscle disease, despite expression of the mutant proteins in skeletal muscle.¹⁴⁸ However, there are mutations in sarcomeric pro-

teins that do cause muscular dystrophy and other myopathies.¹⁴⁹ In some cases, it appears as though muscular dystrophy mutations in sarcomeric proteins do not cause significant heart disease. Therefore, with sarcomeric proteins it appears as though different mutations in these proteins may produce markedly different cardiac- or skeletal muscle-specific phenotypes depending on the location of the mutation and the functional or structural property of the protein that is affected.

Tibial muscular dystrophy/LMGD 2J (titin)

Titin is a huge protein that spans and binds to the Z-line and the M-line of the sarcomere in striated muscle. Titin is thought to be important in maintaining sarcomere structure and the elasticity of the sarcomere.¹⁵⁰ Mutations in the gene encoding titin have been linked to dilated cardiomyopathy.¹⁵¹ However, mutations in titin have also been linked to tibial muscular dystrophy/LGMD2J.¹⁵² As its name implies, tibial muscular dystrophy, primarily affects the tibialis anterior, causing patients to have 'foot drop'. Mutations in titin have also been linked to muscular dystrophy in a spontaneous mutant mouse, the *mdm* or *muscular dystrophy with myositis* mouse.¹⁵³ There have been neither cases of cardiomyopathy in large studies of tibial muscular dystrophy, nor reports of cardiomyopathy in the *mdm* mouse.¹⁵² Although the molecular basis for the tissue specificity of titin mutations is unclear, all the mutations that cause muscular dystrophy reside near the calpain-binding site near the M-line on titin, while mutations that cause cardiomyopathy reside primarily near the Z-line region of titin.¹⁵⁴ Therefore, the interactions of titin and calpain may be important in the muscular dystrophy phenotype of titin mutations. In fact, tibial muscular dystrophy patients show a secondary loss of calpain 3 in skeletal muscle.¹⁵⁵

Limb girdle muscular dystrophy 2A (calpain 3)

Calpain 3 is a member of family of calcium-activated proteases and is expressed in adult skeletal muscle. Although, not typically thought of as a sarcomere protein, as described above calpain 3 is known to bind to sarcomeric titin.¹⁵⁶ Patients with

limb girdle muscular dystrophy 2A have recessive mutations in calpain 3 leading to a loss of calpain 3 activity.¹⁵⁷ Although calpain 3 is expressed in fetal cardiac muscle during cardiogenesis, the protein expression declines to non-detectable levels in the adult heart.¹⁵⁸ Therefore, the skeletal muscle-specific expression of calpain 3 may explain the lack of cardiovascular phenotypes in muscular dystrophy patients with mutations both in calpain 3 and in titin.

Limb girdle muscular dystrophy 2G (telethonin)

Telethonin (also called T-cap) is a small protein that binds to titin and other Z-line proteins such as α -actinin, at the Z-line.¹⁵⁹ Recessive mutations in telethonin result in loss of telethonin expression and limb girdle muscular dystrophy 2G, with an interesting feature of rimmed vacuoles within the muscle.¹⁶⁰ Telethonin is expressed in both skeletal and cardiac muscle, and cardiovascular involvement has been reported in one family with half of the members showing cardiovascular symptoms.¹⁶⁰ Telethonin interacts with titin near the sites that are mutated in DCM, and also interacts with muscle LIM protein (MLP).¹⁶¹ Telethonin has been proposed to be a mechanical stress sensor in cardiac muscle along with MLP, and mutations in MLP also cause both HCM and DCM.^{161,162}

Limb girdle muscular dystrophy 1A (myotilin)

Myotilin is expressed in both adult cardiac and skeletal muscle, binds to α -actinin, and is believed to help cross link actin filaments at the Z-line.¹⁶³ Myotilin is more broadly expressed during development, and may be involved in actin filament assembly in other tissues. Mutations in the gene encoding myotilin have been linked to autosomal dominant limb girdle muscular dystrophy 1A.¹⁶⁴ Patients typically present with proximal muscle weakness and a characteristic dysarthric pattern of speech.¹⁶⁴ Despite the expression of myotilin in the heart, no cardiac involvement has been reported in LGMD1A, even though large families with this disorder have been characterized.¹⁶⁵

Nuclear membrane proteins

Like the plasma membrane, the nuclear membrane also has a submembrane network of proteins that are involved in structure and function within the nucleus. A number of mutations in nuclear membrane proteins have been linked to a variety of cardiovascular and muscle diseases. These genetic studies have helped to define the important role of the nuclear membrane in cellular function, both in muscle and in other tissues. Most of the muscular dystrophy patients with genetic defects in nuclear membrane proteins present with cardiovascular phenotypes. However, as described below, in some cases the various disorders associated with the mutation in the same protein appear to only have partial clinical overlap.

X-linked Emery-Dreifuss muscular dystrophy (emerin)

Emerin is expressed underneath the nuclear membrane.¹⁶⁶ Emerin appears to interact directly with the nuclear membrane and a protein called BAF. BAF in turn binds to heterochromatin.¹⁶⁷ In addition, a protein called nesprin appears to cross link emerin and lamin A/C, and help to form a subnuclear membrane cytoskeleton.¹⁶⁸ Therefore, it is still not clear whether or not the primary function of emerin is to regulate gene expression through an interaction with chromatin, or to regulate nuclear membrane structure. Mutations in emerin have been linked to X-linked Emery-Dreifuss muscular dystrophy (XL-EDMD).¹⁶⁹ Mutations in the gene encoding emerin tend to either cause the loss of stable protein expression or mislocalization of emerin from the nucleus.^{170,171} XL-EDMD is characterized by early contractures (prior to muscle weakness), a slowly developing progressive muscle weakness that affects the proximal upper arms and distal lower limbs, and significant cardiac conduction defects. The cardiac defects are the most serious clinical aspect of XL-EDMD with sudden death being common from complete heart block or other forms of arrhythmia.

Longitudinal studies of XL-EDMD patients have been described, and conduction system defects are very common.¹⁷² Conduction defects usually first present as prolonged PR interval and

sinus bradycardia. Complete sinoatrial and atrioventricular block, atrial flutter or complete paralysis are common, in some cases without any patient-perceived symptoms. In many cases the atria become dilated, and in some cases patients develop dilated cardiomyopathy, heart failure or sudden death.^{172,173} Because conduction defects and sudden death are common in XL-EDMD, 24-hour Holter monitoring is recommended, and pacemaker insertion can be life saving.¹⁷² Female carriers of emerin mutations do not have significant skeletal muscle disease, but can present with cardiac conduction defects.^{174,175}

Autosomal dominant Emery–Dreifuss muscular dystrophy and limb girdle muscular dystrophy 1b (lamin A/C)

Lamin A and lamin C are broadly expressed, and are proteins produced from the same gene (Lamin A/C, LMNA) but differ in their C terminus.¹⁷⁶ As mentioned above, lamin A/C are components of intermediate filaments that reside underneath the nuclear membrane and form the submembrane cytoskeleton. Mutations in lamin A/C have been described in autosomal dominant Emery–Dreifuss muscular dystrophy (AD-EDMD),¹⁷⁷ and autosomal dominant limb girdle muscular dystrophy 1B (LGMD1B).¹⁷⁸ AD-EDMD is clinically similar to XL-EDMD, with early contractures, humeral-peroneal weakness and cardiac conduction defects. LGMD1B is clinically defined by the absence of early contractures, and slowly progressive limb girdle weakness, with cardiac conduction defects. The complexities of variable clinical phenotypes from mutations in the same gene are confounded by the fact that mutations in LMNA can also cause DCM without muscular dystrophy,¹⁷⁹ familial partial lipodystrophy,^{180,181} autosomal recessive Charcot–Marie–Tooth syndrome type 2B1,¹⁸² mandibuloacral dysplasia (MAD),¹⁸³ atypical Werner's syndrome,¹⁸⁴ and Hutchinson–Gilford progeria syndrome.^{185,186} Although many of these disorders are considered to be clinically distinct, there can be overlapping clinical features. For example, MAD patients also have partial lipodystrophy,¹⁸³ and DCM patients with LMNA mutations also have cardiac conduction defects.¹⁷⁹ Recently a mutation in a single family has been

described that has features of Charcot–Marie–Tooth syndrome, muscular dystrophy, heart conduction defects and DCM.¹⁸⁷

The molecular basis of the different clinical features of LMNA mutations is currently unclear. Originally, it was believed that perhaps different mutations in LMNA affect separate functions or interactions of lamin A/C in a genotype/phenotype-specific manner. However, experimental evidence and sufficient genetic evidence for this hypothesis is lacking. As described above, certain genetic loci can display features of several disorders. Furthermore, LMNA mutants with mutations representing different disease mutations, appear to have no consistent genotype/phenotype dependent effect on levels of protein expression or localization.¹⁸⁸ The most striking example of experimental evidence for the lack of genotype-specific effects on lamin A/C function is a knock-in mouse where a specific mutation was knocked in to the LMNA locus to represent a model of AD-EDMD.¹⁸⁹ Heterozygous mice do not display any evidence of muscular dystrophy like human patients, but homozygous mice display a phenotype consistent with Hutchinson–Gilford progeria syndrome. Therefore, there appears to be a relative continuum of clinical entities that can be caused by lamin A/C mutations. Depending on the severity of the mutation, whether the mutation is homozygous or heterozygous, and probably the genetic background, certain clinical phenotypes are more prominent or apparent than others.¹⁹⁰

How LMNA mutations cause disease, particularly in skeletal and cardiac muscle is currently unclear. As with emerin, it is unclear whether or not lamin A/C primarily functions in nuclear structure or regulation of gene expression. Knockout mice for lamin A/C have been generated and display a severe EDMD phenotype and die early.¹⁹¹ Cells from lamin A/C null mice, and some human EDMD patients show some rare evidence of nuclear membrane damage.^{191–193} However, lamin A/C is also localized into speckles that appear to be involved in spatial organization of gene transcription and splicing.¹⁹⁴ Alternatively, it may be that the structural and gene regulation hypotheses are not mutually exclusive. Recently, lamin A/C null cells, when subjected to external mechanical

strain, show increased nuclear deformation and decreased nuclear factor- κ B (NF- κ B) transcriptional response or alterations in other transcription factor localization.^{195,196} Therefore, both nuclear membrane structure and transcriptional responses to external mechanical strain may be altered by LMNA mutations, and may be involved in some of the disease processes in striated muscle where changes in mechanical strain of the cells is frequent.

Cytosolic proteins

Limb girdle muscular dystrophy 2H (TRIM 32)

TRIM32 is a member of the tripartite motif family of proteins of which there are more than 37 family members.¹⁹⁷ Mutations in TRIM family members have been identified in a number of human diseases. TRIM32 is ubiquitously expressed in the cytosol of cells, and is believed to be involved in the ubiquitin-proteasome pathway.¹⁹⁸ Mutations in TRIM32 have been identified in autosomal recessive LGMD2H, a mildly progressive limb girdle muscular dystrophy primarily within the Manitoba Hutterite population.¹⁹⁹ There is no cardiovascular involvement reported in LGMD2H.

Nucleotide repeat disorders

Nucleotide repeats with varying length and complexity are quite common within the human genome. In some cases the repeats occur within coding regions of genes, but often they reside in non-coding sequence. Several human diseases have been linked to alterations in nucleotide repeats including fragile X syndrome and Huntington's disease.²⁰⁰ Alterations in nucleotide repeats have also been linked to forms of muscle disease.

Facioscapulohumeral muscular dystrophy

Facioscapulohumeral muscular dystrophy (FSHD) has been linked to deletions of an integral number of tandem 3.2 kb repeats within a region of chro-

mosome 4 known as D4Z4.^{201,202} Normal individuals have between 11 and 150 repeats while <11 repeats is associated with FSHD.²⁰³ FSHD is an autosomal dominant disorder that often produces asymmetrical weakness affecting the muscles of the face, upper back and arms. The molecular mechanism of FSHD is unclear, but recent evidence suggests that the D4Z4 region might be a transcriptional repressor of nearby genes on chromosome 4, and the repeats may be a binding site for transcriptional repressor factors.²⁰⁴ Cardiovascular involvement is uncommon in FSHD, but in rare cases clinically significant ECG abnormalities or induced ECG abnormalities have been detected.²⁰⁵

Myotonic dystrophy

Myotonic dystrophy is one of the most common forms of muscular dystrophy in humans with a frequency of about 1 in 8000 live births.²⁰⁶ Two forms of autosomal myotonic dystrophy have been described. Myotonic dystrophy type 1 (DM1) has been linked to a trinucleotide repeat expansion within the 3' untranslated region of myotonic dystrophy protein kinase gene (DMPK).²⁰⁷⁻²⁰⁹ In normal individuals, the (CTG) n repeat length is $n = 5-34$, but raises to $n = 50-2000$ in DM1. DM1 has a number of clinical symptoms including active myotonia (which may have both muscle and peripheral nerve components), central nervous system defects, cataracts, endocrine abnormalities including diabetes, gastrointestinal tract dysfunction and cardiac involvement.⁷ Myotonic dystrophy type 2 (DM2) shares many clinical features with DM1, and has been linked to an expansion of a (CCTG) n repeat within the first intron of the gene encoding zinc finger protein 9.²¹⁰

The cardiovascular complications in myotonic dystrophy are significant, and sometimes the earliest signs of disease.⁷ The most common findings in DM1 are cardiac conduction defects in approximately 65% of patients.²¹¹ The mean age of death is 53 years in DM1 patients, with 30% of fatalities being due to cardiovascular events, and 40% due to respiratory failure. Upon ECG monitoring, patients show long PR intervals, wide QRS and late potentials.²¹² Ventricular arrhythmias, as well as atrial flutter or fibrillation are also quite common, sometimes resulting in clinical symptoms.²¹³ Malignant ven-

tricular tachycardia may also be a significant cause of sudden cardiac death in DM1 patients, and require treatment in addition to pacemaker implantation.²¹⁴ Direct myocardial dysfunction is not frequently observed, but in some cases of cardiac hypertrophy, diastolic dysfunction, ischemic heart disease and mitral valve prolapse have all been described in DM1 patients.²¹⁵ A recent large-scale retrospective study suggests approximately 14% of patients have detectable left ventricular systolic dysfunction, while only 2% received a diagnosis of heart failure.²¹⁶ Some studies suggest significant coronary vascular abnormalities may precede the development of cardiomyopathy in DM1 patients.²¹⁷ Autopsy analysis indicates that the conduction system and ventricular myocytes are both common targets for pathogenic processes.²¹⁸ Although not as extensively studied, DM2 patients also have a high prevalence of cardiac conduction defects.²¹⁹

The exact molecular basis of myotonic dystrophy is currently unclear, but the field is developing rapidly. The first myotonic dystrophy hypothesis was that the nucleotide expansion in DM1 may be causing either abnormal expression of DMPK itself, or a heterochromatin-like silencing effect on neighboring genes. The *DMPK* gene was therefore disrupted in mice,^{220,221} Mice developed a mild myopathy with no typical signs of myotonic dystrophy such as skeletal muscle weakness. However, *DMPK* homozygous knockout mice do have cardiac conduction defects, and the severity of the defect seems to be related to the gene dosage of *DMPK*, because heterozygous mice have a less severe cardiac conduction defect.²²² To test the potential effects of the expansion on neighboring gene expression, the *Six5* gene was knocked out in mice, and heterozygous and homozygous mice have cataracts,^{223,224} so *DMPK* activity and *Six5* may play a role in some aspects of myotonic dystrophy. However, this could not explain how an expansion on another chromosome could result in a similar myotonic dystrophy (DM2) and cardiac conduction defects. Most of the recent data to date indicate that a majority of the myotonic dystrophic phenotype can be ascribed to a toxic gain of function of mutant mRNA containing expanded repeats. These repeat-containing mRNAs tend to

cluster within nuclei and sequester nuclear factors, such as a CUG/CCUG-binding protein called muscleblind, that are required for gene transcription regulation and gene splicing machinery.²²⁵⁻²²⁷ Several muscle genes have been shown to be alternatively spliced in myotonic dystrophy such as the insulin receptor and *ClC-1* chloride channels.^{228,229} In support of this latter hypothesis, transgenic mice overexpressing expanded CUG repeat mRNAs, and the muscle-blind knockout mouse both have many of the features of myotonic dystrophy.^{230,231} Furthermore, a transposon insertion in the *ClC-1* gene causes myotonia in mice.²³² Therefore, some of the myotonic dystrophy cardiovascular phenotype may be due to a loss of *DMPK* function, but it is likely that abnormal splicing also plays a role in disease phenotypes and cardiac conduction defects in both DM1 and DM2 patients.

Oculopharyngeal muscular dystrophy
Oculopharyngeal muscular dystrophy (OPMD) is inherited in both an autosomal dominant and recessive pattern, and patients present with pharyngeal weakness and ptosis of the eyelids.²³³ OPMD has been linked to trinucleotide expansion within the polyadenylate-binding protein nuclear 1 gene.²³⁴ In this muscular dystrophy, the nucleotide expansion is in the coding region of the gene, and encodes for an expanded polyalanine track of 10 alanines (normal) to 11 alanines (recessive OPMD) or 12–17 alanines (dominant OPMD). Mutant PABPN1 appears to accumulate in the nucleus as intranuclear inclusions, and the expanded polyalanines may cause oligomerization of this protein into filaments.²³⁵ No cardiovascular phenotypes have been reported in OPMD patients.

Acquired forms of cardiomyopathy
Muscular dystrophy genes in many cases appear to provide a genetic explanation for relatively rare forms of human cardiovascular disease. However, muscular dystrophy proteins have also been identified as targets for pathological processes within more common forms of acquired cardiovascular disease.

Enteroviral infections are a common cause of human myocarditis. Enteroviral infections trigger a number of cascades within cells to allow for viral

replication. Enteroviral infection, particularly from coxsackievirus B3, has been shown to result in the expression of enteroviral protease 2A that can cleave dystrophin.²³⁶ This dystrophin cleavage results in disruption of the DGC and sarcolemmal integrity of virus-infected cells. Interestingly, when the same coxsackievirus B3 infection is performed in mdx mice, the myocarditis is much worse.²³⁷ Although, this clearly indicates dystrophin is probably not the only pathogenic player in coxsackievirus infection-induced myocarditis, it does suggest dystrophin cleavage may be involved in reducing sarcolemmal integrity to allow viral dissemination and further infection.

In addition, a number of muscular dystrophy proteins appear to be remodeled following ischemia-reperfusion injury. For instance, dystrophin appears to be lost from the sarcolemma following ischemia, and in randomly sampled human heart failure patients.^{238,239} This may be due to some downstream effect of ischemia on the cleavage of β -dystroglycan.²⁴⁰ Some of the changes in DGC protein expression may be explained by the activation of myocardial calpains.²⁴¹ Caveolin 3 also changes its localization from the sarcolemma to the cytosol following myocardial ischemia or experimental myocardial infarction.²⁴² It is not clear if these changes in muscular dystrophy proteins play a causal role in the development of myocardial dysfunction following ischemia, but it is well known that genetic alterations in these proteins can indeed cause cardiovascular phenotypes

Clinical recommendations and therapies for cardiomyopathy in muscular dystrophy patients

Recently, the European Neuromuscular Center (ENMC) has produced a guide for the clinical management of heart disease in muscular dystrophy patients and can be consulted for details.⁹ In specific muscular dystrophies, such as myotonic dystrophy, clinical recommendations from other groups have also been recently published.^{7,243} Even though the cardiovascular complications in some forms of muscular dystrophy are rare, many dystrophies do not have large natural history studies, and

therefore cannot precisely define the prevalence or risk of cardiovascular disease. Therefore, it is probably warranted that all patients with muscular dystrophy receive a cardiovascular examination (ECG and echocardiography) at the time of diagnosis, especially if the genetic diagnosis is unclear. In addition, the ENMC recommends routine cardiovascular follow-up in nearly all patients with muscular dystrophy at regular intervals: annually for DMD and sarcoglycanopathy patients over age 10, every two years for DMD and sarcoglycanopathy under age 10, and every 5 years for BMD patients or DMD carriers after the age of 16. In cases where cardiac conduction defects are common (XL-EDMD, AD-EDMD/LGMD1B, myotonic dystrophy) rigorous 12-lead ECGs should be performed at diagnosis, and annually afterwards.

As is the case with skeletal muscle disease in muscular dystrophy patients, there are no direct therapies defined that will completely prevent or cure cardiovascular disease in muscular dystrophy patients. Standard care for heart failure treatment applies in muscular dystrophy cases where there is a progressive trend toward cardiomyopathy or heart failure. Angiotensin-converting enzyme (ACE) inhibitors and sometimes β -blockers, depending on symptoms, appear to be beneficial in improving symptoms in DMD/BMD and sarcoglycanopathies. Although verapamil and nifedipine have been used in the sarcoglycanopathy mouse and hamster to prevent cardiomyopathy, no trials of the efficacy of agents, such as calcium channel blockers, targeting coronary vasospasm have been reported in human patients, so their use should be approached with some caution. In cardiomyopathies with conduction defects, there are no clear indications that anti-arrhythmic drugs are effective. Pacemakers are highly recommended in myotonic dystrophy and XL-EDMD when conduction defects are progressive, even if there are no clinical symptoms. However, in laminopathies (AD-EDMD/LGMD1D) pacemakers may not be the method of choice. Dilated cardiomyopathy can occur in laminopathy patients in concert with ventricular conduction defects. Sudden death is often seen even with pacing.²⁴⁴ Therefore, implantable defibrillators are currently generally recommended in patients with laminopathies. Cardiac transplan-

tation has been performed and is a viable alternative in some cases of BMB, XLDCM and DMD carriers, where cardiovascular symptoms are severe but skeletal muscle function is less affected or normal.³

Primarily due to a lack of understanding of the molecular mechanisms underlying inherited cardiomyopathies in muscular dystrophy patients,

there are a number of groups trying to develop genetic therapies to directly restore normal proteins to dystrophic skeletal muscle or the heart. Approaches include gene transfer vectors, oligonucleotide-directed genetic repair, and myoblast- or stem cell-mediated cell therapies. There are a few reports using adenovirus or adeno-associated virus to deliver mini-dystrophin genes or normal δ -

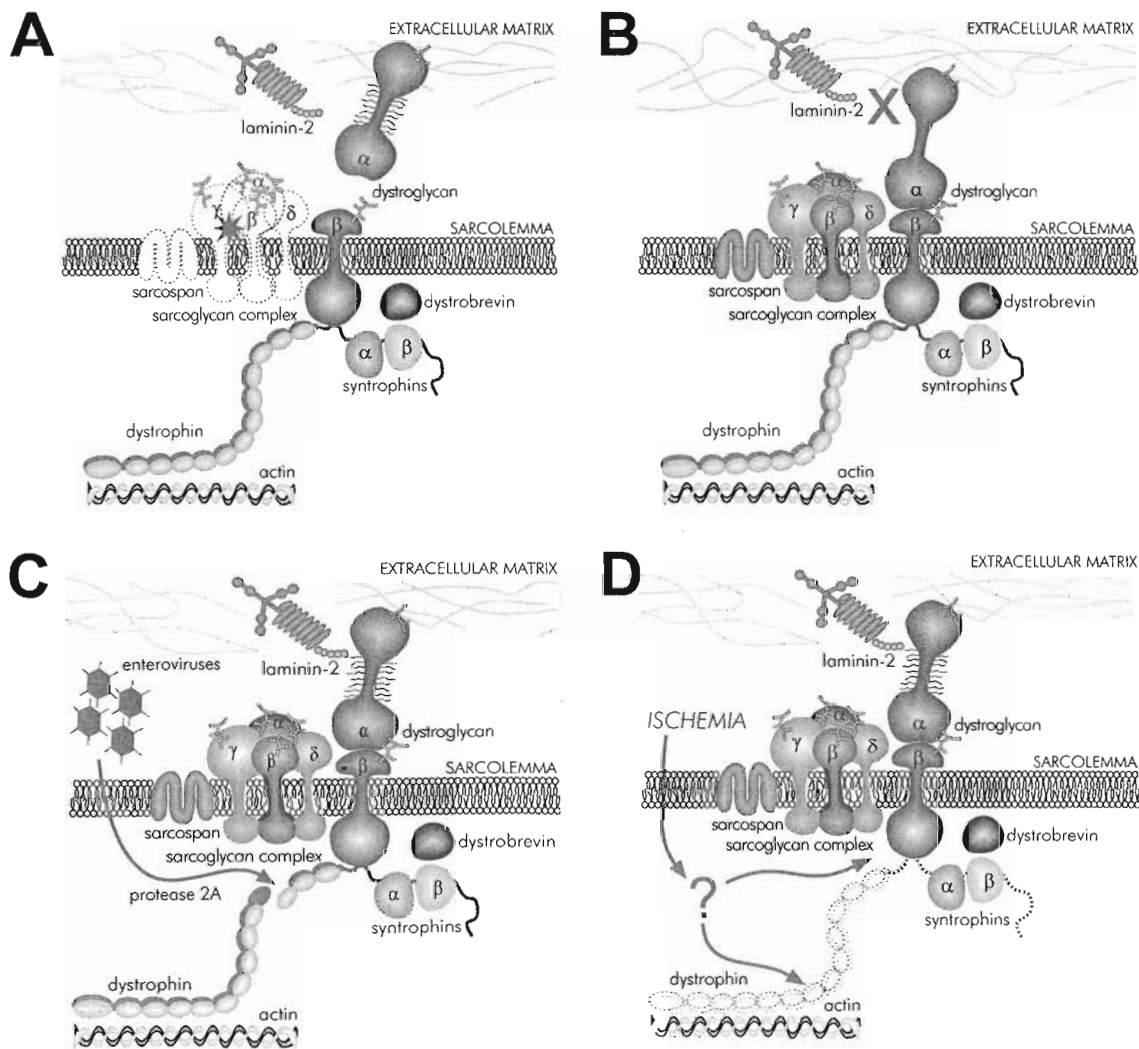


Figure 32.3. Different pathways to disruption of the dystrophin–glycoprotein complex in the heart. Dystrophin–glycoprotein complex function can be disrupted: (A) by direct mutation of its components, (B) secondary to the loss of enzymes required for dystroglycan function, (C) secondary to enteroviral infection, and (D) secondary to myocardial ischemia–reperfusion injury.

sarcoglycan to the heart of the mdx mouse or the cardiomyopathic hamster, respectively.²⁴⁵⁻²⁴⁷ The preliminary results are encouraging and seem to show some limited or significant effect on restoring sarcolemmal integrity. However, full prevention of functional cardiomyopathy has not been demonstrated. In addition, gene transfer of therapeutic proteins targeting calcium handling or β -adrenergic receptor signaling within cardiac myocytes have been tested in the cardiomyopathic hamster, and seem to be able to improve cardiac function.^{248,249} Issues of gene vector delivery, vector serotype, efficiency and efficacy are currently being examined in a number of animal models.

Summary

Clearly, a number of advances in our understanding of the genetic basis of human cardiomyopathy have come from the genetic, molecular and physiological studies on muscular dystrophy patients and mouse models. The complexity of the molecular mechanisms are beginning to be realized as important muscle proteins can be affected by direct genetic mutation, mutations in proteins or mRNAs that bind to or modify proteins (such as glycosylation enzymes), and non-genetic pathways (such as viral infections and changes in extracellular environment due to other systemic diseases) (Fig. 32.3). Hopefully, by understanding these complex molecular pathways, the most critical proteins and pathways that lead to muscular dystrophy and its associated cardiomyopathies will be identified. With advances in patient care, particular respiratory care, muscular dystrophy patients are living much longer today than in the past, and cardiovascular disease will probably become an even more important issue in the clinical care of muscular dystrophy patients.²⁰ In addition, the molecular understanding of these relatively rare genetic disorders will hopefully define critical pathways that may be involved and targeted in more common acquired forms of muscle and cardiovascular disease.

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