GENOTYPE-PHENOTYPE ASSOCIATION ANALYSIS
OF DILATED CARDIOMYOPATHY
IN BECKER MUSCULAR DYSTROPHY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
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The Ohio State University
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ABSTRACT

**Background:** Becker muscular dystrophy (BMD) is caused by dystrophin gene mutations resulting in skeletal and cardiac muscle degeneration. When cardiac manifestations are disproportionate to skeletal muscle involvement, the disorder is diagnosed as X-linked dilated cardiomyopathy (XLDCM). Dystrophin mutations predictive of dilated cardiomyopathy (DCM) have been suggested, but representative examples from the literature are insufficient in number to establish with certainty. Defining the cardiac presentations of specific mutations is crucial for risk classification, early detection and treatment of DCM to prolong survival. From the patient’s perspective, knowing how his individual genotype affects cardiac outcome may be beneficial for health management and life planning.

**Purpose:** To determine age of DCM manifestation in relation to dystrophin genotype and its resulting dystrophin protein modifications in BMD and XLDCM patients.

**Methods:** BMD and XLDCM patients with dystrophin exon deletion mutations and with cardiac histories were collected from multiple sources. The correlations between dystrophin genotypes and cardiac phenotypes were established. Protein modeling was conducted to evaluate structural consequences of genetic mutations.
**Results:** Data of 110 patients were included in this study, of which 76 were cardiomyopathic. Exon deletions in the amino-terminal domain of dystrophin resulted in the earliest mean age of cardiac involvement (22.5 years). Mutations affecting the spectrin repeats in the rod domain preserving Hinge 3 lead to the intermediate age of cardiomyopathy onset (29.5 years), followed the latest age of onset (46.5 years) from patients with rod-domain mutations removing Hinge 3 (p < 0.05). Mutations affecting the spectrin repeats preserving Hinge 3 were subdivided based on whether they disrupted (out-of-phase) or preserved (in-phase) the spectrin repeats structure. Out-of-phase mutations predisposed to earlier age of cardiomyopathy onset (25.5 years) compared to in-phase mutations (36.5 years) (p < 0.05). Protein modeling analyses indicated that dystrophin protein structural modification as a result of genetic mutations may underlie the observed earlier onset of DCM in patients with out-of-phase mutations.

**Conclusions:** An association between dystrophin genotype and cardiac phenotype was identified in patients with BMD and XLDCM. The results enable nursing and medical practitioners to provide timely interventions for at-risk patients to optimize cardiac outcome.
DEDICATION

To my family

to my father and mother, Wen Wen-Shi (Mike) and Hsieh Yeh-Jung (Anne), for giving me abundant love, hope and opportunities,

to my sister and brother, Wen Hsing-Hua (Liz) and Wen Po-Hua (Brian), for showing me worlds beyond mine,

and to the love of my life, Brian Kevin Kaspar, for the life-changing laughter, support and true friendship. You are my role model and my motivation.

To my patients

for trusting me as your nurse.
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<td>Becker muscular dystrophy</td>
</tr>
<tr>
<td>CK</td>
<td>creatine kinase</td>
</tr>
<tr>
<td>DAPC</td>
<td>dystrophin-associated protein complex</td>
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<tr>
<td>DCM</td>
<td>dilated cardiomyopathy</td>
</tr>
<tr>
<td>DMD</td>
<td>Duchenne muscular dystrophy</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>ECG</td>
<td>electrocardiogram</td>
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<td>EPSS</td>
<td>E point-to-septal separation</td>
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<td>IRB</td>
<td>Institutional Review Board</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>NCH</td>
<td>Nationwide Children’s Hospital</td>
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<tr>
<td>nNOS</td>
<td>neuronal nitric oxide synthase</td>
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<tr>
<td>ORF</td>
<td>open reading frame</td>
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<td>OSU</td>
<td>The Ohio State University</td>
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<td>OSUMC</td>
<td>The Ohio State University Medical Center</td>
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<tr>
<td>RNA</td>
<td>ribonucleotides</td>
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<td>UDP</td>
<td>United Dystrophinopathy Project</td>
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XLDCM     X-linked dilated cardiomyopathy
CHAPTER 1

INTRODUCTION

Knowledge of genetics is essential to the thorough understanding of human health and disease. The earliest genetic study was done in the 1860’s by Gregor Mendel, who showed that traits of pea plants are passed from parents to offspring in predictable ways that are controlled by some discrete mechanisms. It is now known that those “mechanisms” are genes. Mendel’s work established the foundation of the science of genetics, which is a branch of biology with the emphasis on heredity and variation. Less than a century took place from Mendel’s observation of heredity to the discovery of deoxyribonucleic acid (DNA) as the genetic material in 1944 by Avery, MacLeod and McCarty (Avery, MacLeod, & McCarty, 1944). Scientific advances in genetics research have greatly enhanced our understanding of disease mechanisms and innovative means to develop new therapy and promote health.

In the field of neuromuscular disorders, Kunkel and colleagues reported the important genetic aspect of Duchenne muscular dystrophy (DMD) in 1987. They discovered that mutations in the dystrophin gene cause to abnormal dystrophin protein expression within the myofibril, resulting in the characteristic progressive muscle degeneration seen in boys with DMD (Koenig et al., 1987). Subsequent discoveries revealed that mutations in the dystrophin gene also caused other allelic disorders,
including BMD and dystrophin-related XLDCM. The development of DCM as part of the disease natural history in these patients has also received much recent attention due to its debilitating impact on the patient’s length and quality of life.

Understanding the genetic pathogenesis in dystrophinopathy has impacted nursing science as nursing research studies take different perspectives to address relevant issues such as newborn screening (Parsons, Clarke, Hood, Lycett, & Bradley, 2002), timing of genetic diagnosis (Parsons, Clarke, & Bradley, 2004) and family function (Chen & Clark, 2007). As an extension, this dissertation research investigated the effect of genetics on health and disease in the context of patients with dystrophin gene mutations. Specifically, this study was a genotype-phenotype correlation analysis in BMD and XLDCM patients. **The study purpose was to elucidate whether there were particular dystrophin genetic mutations that predisposed patients to early- versus late-onset DCM.** This research pursuit is of special relevance to clinical practice, since at the present time practitioners cannot define the BMD and XLDCM patient’s risk of developing DCM in advance due to lack of reliable predictive factors. Consequently, diagnosis and treatment of cardiomyopathy in these populations does not begin until cardiac symptoms are present, leading to poorer prognosis and premature death. From the patient’s perspective, knowing how his individual genotype affects cardiac outcome may be beneficial for health management and life planning.

This dissertation research will be sequentially presented in the following 4 chapters. Chapter 2 is a review of literature. Part I introduces the clinical pictures of DMD, BMD, XLDCM in depth, followed by a discussion of the current understanding of the pathophysiology of DCM in these patients. The focus of Part II is on the molecular

2
and genetic aspects of the dystrophin gene and its protein, and how these mechanisms relate to the clinical phenotypes. Previous genotype-phenotype correlation studies will be reviewed and the rationale of only including BMD and XLDCM patients in this study will be explained. Finally, Part III of this chapter provides the synthesis of literature and states the purpose, the research aims and the theoretical framework underlying this research.

In Chapter 3, the design and methodology of this study are described. This chapter details the procedures of subject collection, inclusion and exclusion criteria, definition and measurement of DCM, grouping of patients, methods used for dystrophin protein modeling and statistical analyses. Chapter 4 reports the results. The findings for each patient group will be discussed, and data addressing each research aim are presented. The final chapter, Chapter 5, discusses the overall meaning and significance of this study. Results of this study will be integrated with current knowledge and implications to clinical practice and future nursing research are concluded.
CHAPTER 2

BACKGROUND

Mutations in the dystrophin genes have been found to be responsible for three distinct clinical presentations described below. Despite the clinical characterization of these three diseases more than a century ago, the discovery of dystrophin and the realization that these three diseases were genetically related have only been recent. For this reason, this chapter will begin with the clinical description of these diseases, followed by a presentation of the genetic mutations that underlie the clinical diversity. The current understanding of the functions of the dystrophin protein in muscle tissues will be discussed, with a particular interest on how genotype influences dystrophin function at a cellular and molecular level leading to phenotypic differences.

Part I: Phenotypes and Clinical Manifestations of Dystrophin Mutation

Duchenne Muscular Dystrophy

DMD is an X-linked neuromuscular disorder with an incidence of one in 3500 live newborn males (A. E. Emery, 1991). It is characterized by progressive, generalized weakness and muscle wasting. The disease was first described at a meeting of the Royal Medical and Chirurgical Society in 1851 by Meryon. He described a familial pattern with a male predilection that ultimately resulted in early death (Meryon, 1851). Twenty
years later, Duchenne, a French neurologist, published a complete histological
description of muscle biopsies from 13 patients. He gave the name “paralysie musculaire
pseudohypertrophique” (pseudohypertrophic muscle paralysis), which accurately
described the muscle paralysis and muscle hypertrophy characteristics of DMD patients
(Duchenne, 1872). The term muscular dystrophy was adopted in 1884 by Erb, who
defined the disease as a primary degeneration of the muscle (Erb, 1884).

Clinically, DMD patients are diagnosed between 3 to 6 years of age with delayed
motor development and skeletal muscle weakness. Due to the X-linked inheritance
pattern, all affected patients are male. Beginning from very early childhood,
approximately half of the affected boys are unable to walk until 18 months after birth, the
age by which normal children have been walking for 6 months. As the boy becomes
older, parents may notice bilateral calf muscle enlargement, frequent falls, inability to run
well or to get up from the floor normally. Mental retardation and/or psychological
disturbances are seen in addition to muscle weakness in 30% of DMD boys (Anderson,
Head, Rae, & Morley, 2002). However, this deficit is not progressive and is not related
to severity of muscle weakness (Giliberto, Ferreiro, Dalamon, & Szijan, 2004). The boy
is usually brought to medical attention when the parents realize that something is
seriously wrong with his motor development. At school age, atypical walking patterns,
including toe-walking, pushing the abdomen forward (lordosis), waddling gait, pelvic tilt,
and difficulty climbing stairs, clearly demonstrate his difficulty in maintaining normal
standing and walking positions. These early hallmarks of DMD develop due to the
combination of the weakness of gluteus maximus and the tightness of hip flexors
(D’Angelo et al., 2009). Progressive pelvic muscle weakness in DMD boys eventually
leads to difficulty in rising from the floor, and their specific way of using other muscles to get up is described as the Gower’s maneuver (Figure 1, Modified from Muscular Dystrophy Association, 2006). In order to get up from the floor, DMD patients face the floor, plant their feet widely apart, raise their buttocks first, and use their hands to “walk” up the legs.

The progressive muscle weakness in DMD boys eventually results in wheelchair dependency at average age 12. Muscle wasting continues to rapidly generalize to other parts of the body, including voluntary and involuntary muscles in the digestive tract, the intercostals muscles and the diaphragm. Interestingly, eye muscles are spared from the degenerative process (A. E. H. Emery, 2008). Common gastrointestinal symptoms include delayed gastric emptying, constipation and impaction (Barohn, Levine, Olson, & Mendell, 1988). The confinement to a wheelchair, in addition to muscle weakness, quickly leads to joint contractures and severe scoliosis. Without appropriate postural support or surgical correction of the scoliosis to prevent aspiration, respiratory insufficiency develops easily particularly for patients who have become bed-ridden. These boys need help with repositioning through the night, often leading to a parent sleeping in the same room. The repositioning request is usually due to respiratory compromise.

Further complicating the clinical picture of DMD is the concurrent development of DCM, a type of cardiomyopathy characterized by enlarged chambers, thinner walls and decreased cardiac pumping ability (Figure 2). Electrocardiographic abnormalities include presence of short PR interval, right ventricular hypertrophy, and inferior-lateral Q waves. These signs are seen in about half the boys and bear no relationship to the
presence of DCM. Many adolescent boys with DMD have increased automaticity characterized by a high average heart rate. Older boys can have supraventricular tachycardia, multiform premature ventricular contractions, ventricular tachycardia or ventricular fibrillation (Thrush, Allen, Viollet, & Mendell, 2009). Pathology followed by corroborative echocardiographic studies on DMD patients with DCM show that the left ventricle myocardial fibrosis and dilation first appear in the left ventricular wall behind the posterior mitral valve leaflet, which progress inferiorly over time toward the apex and into the septum, ultimately affecting the entire left ventricle (Allen, Mendell, & Hoffman, 2008). Despite improvements in respiratory care, most DMD patients die from cardiac failure by 25-30 years of age (English & Gibbs, 2006; Jefferies et al., 2005).

Dilated Cardiomyopathy Pathology in Duchenne Muscular Dystrophy

Many DMD patients develop sinus tachycardia by 5 years of age and conduction changes by 10 years of age. Characteristic irregular conduction patterns seen in a 12-lead electrocardiogram (ECG) non-specific to XLDCM include short PR interval, right ventricular hypertrophy, prolonged QTc interval, and prominent Q waves in leads I, aVL, V5, and V6 or in leads II, III, aVF, V5, and V6 (Thrush, Allen, Viollet, & Mendell, 2009). Shortened PR intervals are seen in about 50% of patients, and QT prolongation is rare. From Holter ECG monitoring, resting sinus tachycardia, loss of circadian rhythm and reduced heart rate variability caused by increased sympathetic activity can be observed (Kirchmann et al., 2005). With advanced fibrosis, more serious arrhythmias may be seen, including atrial fibrillation, atrioventricular block, ventricular tachycardia and ventricular fibrillation (Corrado et al., 2002).
With DMD, myocardial fibrosis and dilation first appear in the left ventricular wall behind the posterior mitral valve leaflet, which over time progress inferiorly toward the apex and into the septum, ultimately affecting the entire left ventricle. Visualization of posterior epicardial thinning and areas of abnormal and/or absent wall movement (e.g. dyskinesis & akinesis), as well as direct measurement of systolic and diastolic dysfunction are possible with echocardiography or other imaging methods such as magnetic resonance imaging (MRI). The present echocardiographic standard established to predict cardiac dysfunction is the ejection fraction; below 55% is considered abnormal in the DMD population (Jefferies et al., 2005; Duboc et al., 2007). Other signs of dilation include increased left ventricular diameter and volume, decreased shortening fraction, and development of mitral valve regurgitation. With echocardiography, the E point-to-septal separation (EPSS) measures the minimal separation between the mitral valve anterior leaflet and the ventricular septum during early diastole. An EPSS of greater than 5-5.5mm suggests that the left ventricle has become more enlarged and can provide a clue for cardiac anatomical changes (Anderson, 2007). Likewise, the sphericity index, calculated by dividing the length of the left ventricle by the width of the left ventricle, is very useful in evaluating the shape of the left ventricular chamber. A sphericity index of greater than 0.66 suggests the presence of DCM, in which the elongated shape of the normal heart becomes spherical (Tani, Minich, Williams, & Shaddy, 2005).

**Becker Muscular Dystrophy**

The allelic variant disorder of DMD is BMD, with symptom manifestations similar to DMD but they appear later and are less severe. The reported incidence for
BMD is at least 1 in 18,450 male live births (Bushby, Thambyayah, & Gardner-Medwin, 1991). The spectrum of clinical presentation and severity of BMD is broader than that of DMD. In general, the BMD patient begins to feel muscle weakness and cramping from his lower limbs at any age during adolescence or adulthood. Toe-walking, waddling gait and lordosis as seen in DMD patients can also be observed in the older BMD patient. There is a wide variation of the severity of muscular degeneration among BMD patients. Some patients become wheelchair-dependent, yet some can function well with light-duty ambulatory aids, such as a cane. Once the BMD patient has become wheelchair-dependent, his risk of developing immobility-related complications is similar to that of the DMD patient. Mental and psychological deficits, while noted in some BMD patients, are present much less commonly than in DMD (A. E. H. Emery, 2008).

Despite the milder skeletal muscle degeneration in BMD, almost all BMD patients still develop DCM (Nigro et al., 2006). Similar to DMD, the severity and onset of cardiac symptoms in BMD patients are unrelated to the degree of skeletal myopathy (Kirchmann, Kececioglu, Korinthenberg, & Dittrich, 2005; Nigro et al., 1995). In fact, DCM is the predominant clinical feature in some BMD cases. Different from DMD, resting tachycardia due to increased automaticity of sympathetic activation is not characteristic in BMD patients (Kirchmann et al., 2005; Vita et al., 2001). Further, decreased ventricular function resulting from myocardial fibrosis in BMD patients can first be seen in the right ventricle. As left ventricular dysfunction progresses, many BMD patients may have symptoms of congestive heart failure since they are ambulatory, in contrast to DMD patients who are largely immobile and asymptomatic. Because of the milder symptoms, most BMD patients live through adulthood and are able to have
children. Their sons will be unaffected, yet their daughters are all carriers who may pass the disease-causing mutation on the X-chromosome to their children. Therefore, families of BMD patients require appropriate genetic counseling for reproductive planning.

*Dilated Cardiomyopathy Pathology in Becker Muscular Dystrophy*

BMD patients have ECG presentations similar to those seen in DMD, including deep Q waves in leads I, aVL (or II, III aVF) and V6, tall R waves in V1 and shortened PR intervals. Bundle-branch block, non-specific ST changes and negative T waves have also been reported (Melacini et al., 1996), as have atrial and/or ventricular arrhythmias such as tachycardia, fibrillation and flutter (Suselbeck, Haghi, Neff, Borggrefe, & Papavassiliu, 2005). However, different from DMD, the Holter ECG in Becker patients does not show the increased automaticity of sympathetic activation (Kirchmann et al., 2005; Vita et al., 2001). The fibrosis in BMD has a similar pattern of progression; however, it often begins in the right ventricular wall. The echocardiographic measurement standards are the same as in DMD. In addition, measurement of the peak systolic and early diastolic strain rates (the rate of deformation of a myocardial segment) may detect abnormal myocardial function not revealed by the measurement of ejection fraction (Meune et al., 2004).

In summary, damage to muscle tissues, particularly respiratory and cardiac muscles, significantly shortens the life expectancy of DMD and BMD patients. With advances in technology, respiratory care for these patients has greatly improved. However, DCM has become the leading cause of death among DMD and BMD patients (Eagle et al, 2002).
**X-Linked Dilated Cardiomyopathy**

In rare cases, mutations in the dystrophin gene are also the cause for some familial XLDCM in which the phenotype is cardiac-specific, generally sparing skeletal muscle. Particularly, study of a group of dystrophin-related XLDCM patients with no detectable skeletal muscle weakness revealed no dystrophin protein expression in the cardiac biopsy but present in the skeletal muscle biopsy (Milasin et al., 1996; Ferlini et al., 1998; Muntoni et al., 1993; Muntoni et al., 1995; Bies et al., 1997). The strong cardiac involvement and mild skeletal muscle phenotype in these patients could be attributed to differences in protein expression between these two types of muscle tissues. However, this explanation cannot be extrapolated to BMD and XLDCM patients with disease-causing mutations in other regions of the dystrophin gene and with proven expression of the mutant dystrophin protein in cardiac muscle (Arbustini et al, 2000; Muntoni et al., 1997; Towbin et al, 1993; Anan et al., 1992; Fanin, Melacini, Angelini, & Danieli, 1999; Ortiz-Lopez, Li, Su, Goytia, & Towbin, 1997; Politano et al., 1999). These cases suggest that some mutations might affect domains of the dystrophin protein critical to its function in cardiac tissue.

**Other Phenotypes of Dystrophin Mutation**

In addition to affecting the function of cardiac and skeletal muscles, dystrophin gene mutations can also be accompanied by the clinical findings in Table 1. The involvement of non-muscle organs agrees with the expression of tissue-specific forms of dystrophin in these tissues, particularly the brain (Davies & Nowak, 2006). Of note, while muscle degeneration is progressive, mental deficits do not worsen over time.
Part II: Mechanisms Underlying the Phenotypes

The Dystrophin Gene

The dystrophin gene, positioned at Xp21.2, is the largest gene known in the human genome, encompassing 2.3 million base pairs of the genomic sequence, which is about 1.5% of the entire X chromosome. The dystrophin gene is comprised of 79 exons and its structure is complex. It contains multiple promoters and regulatory elements in its non-coding regions that drive tissue-specific expression of at least 9 distinct dystrophin protein isoforms of varying size and function (Leiden Muscular Dystrophy pages, 2006). In muscle, dystrophin includes 79 exons that encode a 13.9 kilobases messenger ribonucleic acid (mRNA) transcript (Kunkel, Beggs, & Hoffman, 1989; Mandel, 1989; Manole, 1995).

Dystrophin Gene Mutations

This large size of dystrophin increases the likelihood of the gene to develop sporadic mutations through rearrangement and recombination events. Mutations on dystrophin gene lead to errors when encoding for dystrophin protein, leading to various phenotypic consequences, including DMD, BMD and XLDCM. As described by the “reading frame theory” (Monaco et al., 1988), in most cases, patients whose dystrophin mutations disrupt the translational reading frame have more severe skeletal muscle symptoms (e.g. DMD), and patients whose dystrophin mutations do not disrupt the translational reading frame have milder phenotypes (e.g. BMD). Early studies investigating the pattern of genetic mutations among both DMD and BMD patients have
shown that exon deletion is the most common form of dystrophin mutations (Darras et al., 1988; Forrest et al., 1987). This observation is consistent with later findings indicating that exon deletions are distributed throughout the dystrophin gene in DMD and BMD, large deletions (>10 exons) occur more frequently between exons 10-45, and smaller deletions (<10 exons) around exons 45-55. Deletion mutations at the 5’ and 3’ ends are rarer compared to their occurrence at the center region of the gene (80%) (Prior, 2006). Another type of mutation, exon duplication, is seen among both DMD and BMD patients. Exon duplication mutations in the dystrophin gene are much rarer than exon deletion mutations (~6% of all cases) (Hu, Ray, Murphy, Thompson, & Worton, 1990). In DMD, most exon duplication mutations occur at the 5’ end of the dystrophin gene, versus the central region for BMD cases (Prior, 2006).

Point mutations of the dystrophin gene that evolve as missense or nonsense mutations are also present among both DMD and BMD patients (Lenk et al., 1996; Prior et al., 1996). Point mutations at regions where dystrophin interacts with its neighboring proteins, such as γ-actin, lead to more the more severe DMD phenotype (Prior et al., 1995). Interestingly, among BMD patients collected in this dissertation study, some also have point mutations at dystrophin’s protein-binding regions, providing a hint that there must be other mechanisms involved in determining the BMD patients’ milder symptoms. Indeed, a fascinating group of dystrophin gene mutations has been classified as “splicing mutation” (Roest et al., 1996). Since conventional mutation detection techniques focus on the exons of dystrophin, mutations in some patients cannot be detected. Using the ribonucleotides (RNA)-based approach, small DNA point mutations within the introns (non-coding region) can be detected. DNA sequence change within the intron can change
the normal splicing pattern in transcription, explaining variations in phenotype (Roest et al., 1996). Finally, insertion mutations in the dystrophin gene account for another type of rare mutation, which has been noted in both DMD and BMD (Gurvich et al., 2008; Musova et al., 2006).

Some mutations of the dystrophin gene occur in the non-coding region. However, they still affect the final product of the dystrophin protein, since they may affect the promoters or tissue specific regulatory elements, such as enhancers. In these circumstances, the phenotypic consequence is tissue-specific. Examples of these have primarily been shown in patients harboring genetic mutations in dystrophin’s muscle promoter or first exon regions (Milasin et al., 1996; Muntoni et al., 1996; Muntoni et al., 1993; Muntoni et al., 1995). The heart is the preferentially affected muscle in these patients because the compensatory up-regulation of dystrophin expression from alternative dystrophin promoters that use a different first exon only occurred in the skeletal muscle but not in the cardiac muscle (Bastianutto et al., 2001).

The Dystrophin Protein

Mutations in the dystrophin gene can lead to lack of protein expression or to expression of an altered dystrophin protein, leading to the varied clinical presentations described above. It is therefore important to understand the structure of the dystrophin protein and the functions carried out by its domains. The dystrophin protein in the muscle is a long rod-shaped protein localized under the muscle fiber membrane, or the sarcolemma. As illustrated in Figure 3, the unique position of the dystrophin protein in the muscle fiber makes itself an important link between the intracellular cytoskeleton and
the extracellular matrix during muscle contraction and stretch. Figure 4 is a representation of the dystrophin gene and protein. As shown, there are four major domains of the dystrophin protein: (1) the amino terminal domain (N-terminus, approximately exons 1-8) anchoring to the intracellular cytoskeleton; (2) the rod domain (approximately exons 9-64 and primarily comprised of spectrin-like repeats) that offers flexibility to absorb the impact of muscle contraction; (3) the cysteine-rich domain (approximately exons 65-69) that links the intracellular cytoskeleton to the extracellular matrix; and (4) the carboxyl terminal (C-terminus, approximately exons 70-79), which is less critical for preserving muscle function. These domains are connected by four hinges, which can be thought as joints in a body that allow bending and movements.

In addition to its localization at the muscle fiber membrane, immunostaining studies have indicated that dystrophin is particularly concentrated in myotendinous and myomuscular junctions, which are implicated in the transmission of contractile force from myofibrils to extracellular elements of muscle tissue (Bassett et al., 2003; Samitt & Bonilla, 1990). Myotendinous and myomuscular junctions are attachment sites between the ends of muscle fibers and tendons or serially arranged muscle fibers, respectively, which ultimately transmit the force of muscle fiber contraction to bone.

Pathophysiology in Dystrophinopathies

When the function of the dystrophin protein is compromised due to gene mutations, the sarcolemma is more fragile and susceptible to damage from muscle contractions leading to increased permeability in the membrane. Extracellular calcium enters the muscle fiber more easily, which in turn lead to protein degradation from
calcium-activated proteases (Constantin, Sebille, & Cognard, 2006). Evidence from DMD skeletal muscle biopsies has shown that the level of dystrophin protein in muscle is $\leq 5\%$, much less than the amount of dystrophin found in BMD patients. The increased permeability of the sarcolemma also leads to the release of intracellular muscle proteins from the myofiber, such as creatine kinase (CK). Increased serum level of CK (>35-174 U/L) is a hallmark of muscle damage and is a diagnostic tool of these two types of muscular dystrophy. Serum CK levels vary with the age and clinical progression in DMD and BMD patients, and it can reach values 200 to 300 times higher than normal (Zatz et al., 1991).

Defects in or absence of dystrophin protein in the cardiomyocyte results in DCM through a pathway similar to that described above. Specifically, recent studies have suggested that absence or mutation of dystrophin disrupts the function of membrane ion channels, particularly the sarcolemmal stretch-activated channels, which respond to mechanical stress (Woolf et al., 2006). When cardiomyocytes with deficient or mutant dystrophin stretch during ventricular filling, the stretch-activated channels do not open appropriately, leading to increased influx of calcium (Williams & Allen, 2007). The high intracellular calcium activates calcium-induced calpains, a group of proteases, which degrade troponin I and compromise cardiomyocyte contraction (Feng, Schaus, Fallavollita, Lee, & Canty, 2001; Gao et al., 1997). Calpain-mediated destruction of membrane protein allows more calcium entry. Eventually, the chronic calcium overload leads to the cardiomyocyte death (Raymackers et al., 2003). Figure 5 is a graphic representation of the pathophysiology event in DCM caused by abnormal dystrophin.
Unlike skeletal muscle, cardiac muscle repeatedly contracts at least 86,400 times per day in the adult and more in the child. The calcium fluxes associated with each excitation contraction cycle are thought to accelerate the deterioration process within cardiomyocytes as compared to skeletal myofibers. In addition, calcium buffering and handling are different between cardiac and skeletal muscle. Cardiomyocyte death initiates an inflammatory response during which macrophages migrate to clear the damaged cells and debris. Following macrophage recruitment, fibroblasts invade the damaged area and form scar tissue (fibrosis) in the heart. Fibrotic tissue is very inflexible compared to the normal cardiac tissue and thus renders myocardial contraction inefficient. The fibrosis begins from the left ventricular wall in DMD and from the right and left ventricular walls in BMD, starting in the epicardium and advancing into the endocardium (Frankel & Rosser, 1976). It progressively spreads throughout most of the outer half of the ventricular wall. This pattern of fibrosis is unique to dystrophinopathy. The fibrotic region will gradually stretch, become thinner, lose contractility, and result in DCM. The dilation of the heart increases left ventricular volume, decreases systolic function, and can lead to mitral valve regurgitation, all of which result in decreased cardiac output and hemodynamic decompensation. The cardiac phenotype in each DMD or BMD patient results from the patient’s particular type of dystrophin gene mutation; however, the relationship between genotype and phenotype remains elusive. Figure 6 depicts the continuing pathologic progression of DCM following cardiomyocyte death.
Interactions of Dystrophin

The pathophysiological findings described above relate to the structural and intracellular signaling functions mediated by the direct and indirect interactions of dystrophin with a large number of proteins. Dystrophin is an essential member of the dystrophin-associated protein complex (DAPC) at the sarcolemma (Figure 3) (Yoshida et al., 2000). Dystrophin binds to the intracellular cytoskeleton through $\gamma$-actin filaments at its N-terminus and at the central rod domain around spectrin-like repeats 11-17 (Sutherland-Smith et al., 2003; Winder, Gibson, & Kendrick-Jones, 1995). Although dystrophin binds actin, it does not interact with the actin filaments mediating muscle contraction at the sarcomere. At the distal end, dystrophin is connected to the extracellular matrix through the transmembrane protein $\beta$-dystroglycan, whose carboxy-terminal cytoplasmic domain binds directly to dystrophin and provides a physical link to other proteins in the DAPC, including $\alpha$-dystroglycan, the transmembrane sarcoglycans ($\alpha, \beta, \gamma, \delta, \epsilon$ and $\zeta$ subunits), sarcospan, and laminin.

Furthermore, recent data proposed that dystrophin also binds to the anchoring proteins ankyrin-B and ankyrin-G. In particular, ankyrin-G binds to both dystrophin and $\beta$-dystroglycan and plays a role in restricting them at costameres (sub-sarcolemmal protein assemblies circumferentially aligned in register with the Z-disk of myofibrils) (Ayalon, Davis, Scotland, & Bennett, 2008). The physical arrangement of dystrophin and DAPC creates a transmembrane micronetwork composed of the intracellular actin, the cell membrane and the basal lamina. This structure can be thought as a bolt (e.g. dystrophin-bolt), which functions to protect the sarcolemma from mechanical stress during repeated cycles of muscle contraction and relaxation. Loss of its integrity
produces muscle fiber fragility, susceptibility to myofibril necrosis and eventually
degeneration of the muscle (Davies & Nowak, 2006).

The extreme C-terminus of dystrophin directly interacts with syntrophins (5
known isoforms) and dystrobrevins (at least 3 isoforms and mainly \( \alpha \) in skeletal muscle).
Syntrophins and \( \alpha \)-dystrobrevins have independent interactions with dystrophin and they
also directly bind to each other. Through the syntrophins, the DAPC anchors to signaling
molecules such as neuronal nitric oxide synthase (nNOS) and ion channels (Davies &
Nowak, 2006; Ervasti & Sonnemann, 2008). The crucial role of dystrophin in a number
of sarcolemmal signaling pathways is evidenced by its involvement and interactions with
many signaling proteins, including calmodulin (Anderson, Rogers, & Jarrett, 1996),
calmodulin-dependent kinase (Madhavan & Jarrett, 1999), extracellular signal-regulated
protein kinases (Shemanko, Sanghera, Milner, Pelech, & Michalak, 1995), casein kinase,
p34\(^{cdk2}\), calcineurin (Michalak, Fu, Milner, Busaan, & Hance, 1996), protein kinase A
(Senter, Ceoldo, Petrusa, & Salviati, 1995), and protein kinases C and G (Luise et al.,
1993).

Given that there is no clear relationship between the severity of the DMD and/or
BMD patient’s skeletal muscle wasting and the severity of his DCM, questions have been
raised regarding whether the functional differences between cardiac and skeletal muscle
dystrophin are due to different protein associations. Investigations aimed to address this
issue have utilized animal models. A widely utilized animal model to study
dystrophinopathy is the \( mdx \) mouse, which expresses no dystrophin protein.
Approximately two to three weeks after birth, the \( mdx \) mouse begins to develop rapid,
progressive myopathy until about seven weeks of age. The myopathy and interstitial
fibrosis slows down but continues throughout the mouse’s 2-year life span. Cardiac
phenotype of the mdx mouse is milder than that seen in human patients; however, it does
develop ECG abnormality, ventricular fibrosis and DCM during mid-to-late adulthood
(Chu et al., 2002; Quinlan et al., 2004).

Past studies have found that mdx animals over-expressing altered dystrophin transgenes which consisted of no to minimal rod domain (e.g. micro- or mini-dystrophin)
demonstrated phenotypic rescue only in the skeletal muscle, but not in the cardiac muscle
(Harper et al., 2002). Therefore, it seems that the rod domain of dystrophin may not be
entirely essential for normal skeletal muscle function, yet it is necessary for normal
cardiac function. This result suggests structural and functional differences exist between
skeletal muscle and cardiac dystrophin. Indeed, the cardiac dystrophin protein has been
shown to have unique binding partners. For example, the cardiac sodium channel Na,1.5
associates with cardiac dystrophin through the mediation of α- and β- syntrophins.
Observation in the mdx5cv animals showed that normal cardiac dystrophin is required for
proper functioning of Na,1.5, which maintains physiologic sodium current and regular
conduction (Gavillet et al., 2006). Deficiency of dystrophin in the cardiomyocyte has
been reported to lead to enhanced calcium influx and delayed calcium channel
inactivation due to deregulation to the properties of the dihydropyridine receptors, the L-
type calcium channel and the sarco(endo)plasmic reticulum Ca$^{2+}$-ATPase (Leonoudakis et
al., 2004; Sadeghi, Doyle, & Johnson, 2002; Woolf et al., 2006). Anatomically,
dystrophin has been found to only be present in the T-tubule and microfilaments of the
cardiac, but not skeletal muscle, and there is a subset of cardiac dystrophin that does not
establish interaction with DAPC (Kaprielian, Stevenson, Rothery, Cullen, & Severs,
2000; Meng, Leddy, Frank, Holland, & Tuana, 1996; Peri, Ajdukovic, Holland, & Tuana, 1994).

Functional Biology of Dystrophin

The functional role of dystrophin can be best understood by observing consequences of dystrophin deficiency. The impact of the absence of dystrophin is evidenced by DMD patients’ severe phenotype, even though in the DMD muscle, dystrophin’s functional analog protein, utrophin, has been reported to be up-regulated and forms an utrophin-bolt at the sarcolemma. The micronetwork connecting the basal lamina and intracellular actin held together by the utrophin-bolt is structurally weaker because only a small amount of β-dystroglycan is bound to utrophin. Moreover, there are not as many utrophin-bolts present on the DMD muscle fiber as compared to the number of dystrophin-bolts in the normal muscle (Ishikawa-Sakurai, Yoshida, Imamura, Davies, & Ozawa, 2004; Tanaka, Ishiguro, Eguchi, Saito, & Ozawa, 1991). Consequently, some DAPC proteins are decreased in the DMD muscle. For example, expression of β-dystroglycan and the sarcoglycans is greatly reduced (Mizuno, Yoshida, Nonaka, Hirai, & Ozawa, 1994; Ozawa, Noguchi, Mizuno, Hagiwara, & Yoshida, 1998; Ozawa, Mizuno, Hagiwara, Sasaoka, & Yoshida, 2005), and the syntrophins, nNOS, sarcospan and dystrobrevin are nearly absent (Brenman, Chao, Xia, Aldape, & Bredt, 1995; Metzinger et al., 1997; Ohlendieck et al., 1993).

Due to the fragile structure of the cell membrane, the DMD muscle cell is more prone to rupture at its delicate loci during muscle contractions. The “leaky” muscle cell membrane explains the finding that some cytosolic proteins, such as CK, are found in the
extracellular milieu in patients with dystrophinopathy. The consequence of the leaky membrane is the long-term loss of cytosolic proteins which eventually disturbs nutrition homeostasis and metabolic processes within the cell, while allowing entrance of substances from extracellular fluids, including calcium, which plays a role in activating calpain, a protease known to damage myofibrils in dystrophinopathy (Bodensteiner & Engel, 1978; Bullard, Sainsbury, & Miller, 1990).

The absence of dystrophin in the myofibril also affects the related signaling pathways. Each of the proteins in the signaling pathways described previously regulates cellular defense mechanisms such as anti-apoptotic pathways, stress-response pathways, antioxidant defense pathways and anti-inflammatory pathways, all of which affect muscle death or survival and have been implicated in the pathogenesis of DMD and BMD (Petrof, 2002). For instance, in the normal muscle the signaling protein nNOS physically binds to the DAPC via α-syntrophin and caveolin-3, preferentially localizes to the sarcolemma of fast fibers and functions to catalyze the production of nitric oxide. In the mdx mouse, nNOS expression in the muscle is significantly lower and is mislocalized inside the cytosol. It is speculated that one cause for DMD pathology originates from the misplacement of nNOS, which consequently increases free radical production and susceptibility to inflammation (Brenman, Chao, Xia, Aldape, & Bredt, 1995; Chang, et al., 1996).

In the cardiac muscle, the lack of the dystrophin protein leads to significant functional compromise. Milasin (1996) reported a patient whose skeletal muscle dystrophin was shown immunocytochemically to be reduced in quantity but was normally distributed. However, his cardiac dystrophin was undetectable. This patient
was only 24 years old when he was admitted to the hospital for severe heart failure that had developed within the preceding 6 months. Prior to this event, the patient did not have any skeletal muscle symptoms (Milasin et al., 1996). In another similar case, a young man with no significant skeletal muscle weakness was found to be in severe heart failure when he was 20 years old. Further examinations confirmed the moderately reduced amount of skeletal muscle dystrophin and the complete absence of cardiac dystrophin (Bies et al., 1997). Both of these patients have confirmed dystrophin genetic mutations which resulted in no cardiac dystrophin protein expression (former: a G to T transversion at the first exon-intron boundary that led to a splice-site mutation; latter: duplication of exons 2-7).

The severe cardiac phenotype is not only due to the lack of cardiac dystrophin expression but also to the altered composition of other proteins in the cardiomyocyte as a result of dystrophin’s absence. In the case of the second patient mentioned above, his α-dystroglycan was lost in the heart, thus the linkage between the DAPC and the extracellular matrix is disrupted (Bies et al., 1997). This finding does not suggest that α-dystroglycan is the only protein that is affected by alterations to dystrophin. Rather, it provides a glimpse to the complicated dynamics existing among cardiac dystrophin and other proteins which are uniquely different from skeletal muscle dystrophin.

Structure-Function Relationship of Dystrophin

Transgenic animal studies have shown that deletion of either the N-terminus or the rod-domain actin-binding site resulted in a mild muscular dystrophy phenotype, but deletion of both actin-binding domains created a severe phenotype despite restoration of
DAPC proteins (Corrado et al., 1996; Cox, Sunada, Campbell, & Chamberlain, 1994; Greenberg, Sunada, Campbell, Yaffe, & Nudel, 1994; 760 Warner et al., 2002). These findings suggest that there may be redundancy with two actin-binding domains, and binding to actin from at least one domain is crucial for normal function. At the central rod domain, the unique flexibility and elastic properties has been well recognized as an important biological function of the protein. Interestingly, there have been mild cases of BMD whose dystrophin mutations eliminated most of the rod domain (England et al., 1990; Matsumura et al., 1994). This phenomenon raised the idea of designing a minimally-composed dystrophin construct for gene therapy, such as mini- or micro-dystrophin, as another approach to elucidate the structure-function relationship of the dystrophin protein. Indeed, functional mini- and micro-dystrophin constructs with very little rod domain but with the amino-terminal actin-binding domain have shown that the flexibility of rod domain does not seem absolutely essential for normal functioning of dystrophin in the skeletal muscle; rather, having the actin-binding properties is more important (Harper et al., 2002).

The cysteine-rich domain and the C-terminus are two regions where dystrophin has interactions with many proteins. At the N-terminus end of the cysteine-rich domain is the WW domain, where binding with β-dystroglycan is present. At the C-terminus, the coiled-coil motif forms the binding site for dystrobrevin and syntrophins (Ishikawa-Sakurai, Yoshida, Imamura, Davies, & Ozawa, 2004; Blake, Weir, Newey, & Davies, 2002). The cysteine-rich domain seems to play an essential role for normal dystrophin functioning, since there has been no report of BMD patients with deletions in this region. Even missense mutations predicted in-frame deletions in this region all lead to the severe
DMD phenotype (Beggs et al., 1991; Ishikawa-Sakurai, Yoshida, Imamura, Davies, & Ozawa, 2004; Koenig et al., 1989; Lenk et al., 1996; 763 Goldberg et al., 1998). Different from the cysteine-rich domain, the C-terminus does not seem to be as crucial, as evidenced by mild BMD phenotype resulting from C-terminus mutations (Kimura et al., 2009; Suminaga, Takeshima, Wada, Yagi, & Matsuo, 2004; Patria, Alimsardjono, Nishio, Takeshima, Nakamura, & Matsuo, 1996). Summarizing dystrophin structure-function studies to date, note that most studies have defined the essential components of dystrophin based on exhaustive testing in the skeletal muscle. However, as mentioned earlier, mini- and micro- dystrophin constructs optimized for skeletal muscle do not fully restore cardiac function implying that they lack protein domains that are functional in the myocardium. The two major domains lacking in mini- and micro dystrophins are the rod domain and the carboxyl terminus.

Phasing of Dystrophin Spectrin-Like Repeats

The structural arrangement, also termed phasing, of the dystrophin rod domain also plays a role in maintaining its function. The dystrophin rod domain is composed of 25 degenerate copies of an amino acid sequence with significant homology to the protein spectrin, forming 24 canonical spectrin-like repeats and one truncated repeat which comprises Hinge 3. The structure of dystrophin’s spectrin-like repeats are found in several cytoskeletal proteins and are hypothesized to consist of a series of three α-helix coiled-coil “beads”. For spectrin, it was suggested that each of the three α-helices is similar in length and is connected by a flexible linker sequence, or loop, to form a flexible rod-like domain (for graphic illustration see Figure 7) (Parry, Dixon, & Cohen,
However, for dystrophin, more recent modeling demonstrated that the dystrophin sequence consists of a long contiguous $\alpha$-helical bridge joining the beads, thereby resulting in a nested repeat structure and a relatively rigid backbone (Calvert, Kahana, & Gratzer, 1996; Cross, Stewart, & Kendrick-Jones, 1990). A shorter $\alpha$-helix folds back to stabilize the interaction of adjacent helix segments (Fig. 11A) (Cross et al., 1990; Koenig & Kunkel, 1990). The exon boundaries do not correlate with the physical boundaries of individual spectrin repeats at the protein level. Therefore, deletion mutations that remove different combinations of exons can disrupt the folding pattern of spectrin repeats to different degrees. In $mdx$ mice, which lack dystrophin, over-expression of transgenic dystrophin constructs that preserve the correct nested repeating structure or phasing (in-phase) of spectrin repeats rescued the skeletal muscle dystrophic phenotype better than a dystrophin construct with a disrupted repeat pattern (out-of-phase) (Harper et al., 2002). It has not yet been determined whether the phasing of spectrin-like repeats is similarly important for the function of dystrophin in human hearts.

In summary, it is clear that the role of dystrophin is structurally and functionally complex in the muscle fiber. For a less severe muscular phenotype in animal models and humans due to dystrophin mutations, it seems that keeping at least one binding site with actin, preserving the cysteine-rich domain and the C-terminus, as well as maintaining the phasing pattern of the rod domain are important.
Genotype-Phenotype Correlations

The size of dystrophin gene deletion mutation does not have absolute correlation with the severity of the resulting phenotype, since there are DMD patients with small deletions and BMD patients with large deletions. Rather, the amount of dystrophin protein produced appears to play a more important role. Our current understanding of this paradox is heavily founded upon the “reading-frame” rule. The genetic and phenotypic differences between DMD and BMD were first delineated by Monaco and colleagues (1988). Immunohistochemical studies of muscle biopsies from more severe patients, most of whom had DMD, had shown the high prevalence of dystrophin protein deficiency or truncation, whereas at least partially functional dystrophin protein was found in patients with less severe symptoms (e.g. BMD). Further examinations of the dystrophin genotypes linked dystrophin protein deficiency/truncation to mutations that disrupted the translational open reading frame (ORF), and partial dystrophin protein expression/function to mutations that preserved the ORF. Therefore, the “reading-frame theory” was proposed (Monaco, Bertelson, Liechti-Gallati, Moser, & Kunkel, 1988). The reading frame theory contends that due to an eventual stop codon, the frame-shifted mRNA would prematurely terminate amino acid synthesis during translation, producing a fragmented dystrophin protein that is likely to be non-functional or degraded. On the other hand, deletions that still maintain the mRNA ORF for translation of amino acids would produce shorter, lower molecular weight dystrophin molecules that are presumably semi-functional.

This theory held true in 92% of the cases in a later study involving 258 patients with dystrophin exon deletion mutations (Koenig et al., 1989). In the Leiden Open
Variation Database, established by the Leiden University in Netherlands as a registry of
dystrophin mutations found worldwide (Fokkema, den Dunnen, & Taschner, 2005), the
reading-frame theory held true 91% of the time in 4700 cases of DMD and BMD
(Aartsma-Rus, Van Deutekom, Fokkema, Van Ommen, & Den Dunnen, 2006). The 8-9%
of the cases for which the reading-frame theory does not explain phenotype has been
expanded to 30% in a recent study (Kesari et al., 2008). Of 56 BMD patients with
deletion or duplication mutations in this study, 17 demonstrated frame-shift mutations,
challenging the well-believed reading-frame logic. Exceptions to the reading-frame
theory may be due to alternative splicing of exons or other epigenetic mechanisms. In
other words, genetic factors can explain the clinical course of a DMD or BMD patient to
a great extent according to established research. However, it is important to note that
exceptions do exist. Thus, in the clinical setting, each patient should be assessed and
counseled on an individual bases.

The correlation of DCM to specific dystrophin genotypes is a challenging one.
The fact that protein is the biological basis for cellular function precludes DMD patients
as appropriate subjects for this type of study. Despite the wide spectrum of dystrophin
mutations among DMD patients, the common feature among them is the near complete or
complete lack of dystrophin protein in the muscle fiber, leading to the relatively
homogeneous phenotypes which define this disease. It is difficult for genotype-
phenotype analyses conducted in the DMD population to be informative, since in general
all genotypes result in the same phenotype. On the other hand, most, but not all, patients
with BMD or dystrophin-related XLDCM represent a group of individuals who have
various dystrophin gene mutations, who do produce dystrophin proteins that reflect their
unique dystrophin genotypes, and who likely present different clinical pictures of the disease as a consequence of their genotypes. This group of patients is more suitable for the research of dystrophin genotypes and resulting cardiac phenotypes since their phenotypes can be explained by both their dystrophin protein make-up and genotypes.

Given the low prevalence of BMD patients and the even rarer cases of dystrophin-linked XLDCM, past genotype-phenotype correlation studies have typically suffered from low numbers of patients that display a large range of genetic mutations. For example, a few genotype-phenotype studies have been performed in BMD patients in an attempt to correlate sites of dystrophin mutations with the occurrence and/or severity of cardiomyopathy. From these studies a possible correlation between DCM development and deletions of exons 48 and 49 of the dystrophin gene was suggested (Maeda et al., 1995; Melacini et al., 1993; Nigro, Politano, Nigro, Petretta, & Comi, 1994). In addition to small numbers of subjects (e.g. 31 subjects in Melacini et al. (1993) study), most patients enrolled in these studies have mutations involving exons 48 and 49, thus mutations elsewhere along the dystrophin gene were significantly under-represented. No correlation of cardiomyopathy was found with mutations affecting the 5' coding region of the dystrophin gene (Melacini et al., 1993; Nigro et al., 1994). However, this is a region found to strongly associate with dystrophin-related XLDCM in other studies (Bies et al., 1997; Feng, Yan, Buzin, Towbin, & Sommer, 2002; Gold et al., 1992; Milasin et al., 1996; Muntoni et al., 1993; Muntoni et al., 1995; Novakovic et al., 2005; Saotome, Yoshitomi, Kojima, & Kuramochi, 2001). Further, upon re-analysis of the Melacini (1993) study as part of the preliminary research for this dissertation study, it was found that some patients classified as having cardiac involvement by the study’s investigators
showed no abnormalities in echocardiogram measurements such as ejection fraction, but primarily had electrocardiogram abnormalities. For this dissertation study, cardiac involvement was defined based upon abnormalities in echocardiogram rather than electrocardiogram, which in dystrophinopathy reflects cardiac function poorly (Corrado et al., 2002; Thrush et al., 2009).

Of relevance, findings from a recent study associated more severe skeletal muscle symptoms with deletion mutations that include exons 45-49 but exclude the adjacent exons 51-52 (Carsana et al., 2005). Such a correlation had not been observed in the two previous studies since patients with both large and small deletions were inappropriately pooled together. The pooling strategy was unfortunately dictated by the limited number of available patients; consequently, less common deletions affecting other regions of dystrophin have been left unstudied. Another recent genotype-phenotype correlation study included 7 BMD and 62 DMD patients (Jefferies et al., 2005). They found a significant association of cardiomyopathy with mutations affecting exons 12 and 14 to 17, as well as a possible protection conferred by mutations in exons 51 to 52. Unfortunately, this study appears to have pooled DMD and BMD patients in order to achieve statistical significance at these loci. This method is arguable since as stated before, DMD patients do not usually express dystrophin and therefore while their genotype may vary, any correlation to a phenotype in the absence of a produced protein is difficult to interpret.
Part III: Dissertation Research

Synthesis of Literature

Dystrophin is an important protein present in muscle tissue. Mutations in the dystrophin gene give rise to DMD, BMD and XLDCM. Evidence suggests that mild phenotypes of these disorders are associated with the mutant dystrophin protein binding with actin, maintaining the cysteine-rich domain and preserving proper phasing of the spectrin-like repeats of the rod domain. DCM is the primary cause of death among most BMD patients. One of the aforementioned studies (Nigro et al., 1994) followed 68 BMD patients over a 17-year period and reported that 100% of patients had either preclinical or clinical cardiac involvement by 30 years of age, regardless of dystrophin genotype. This report suggests that perhaps the most important question to ask in research is not whether each patient’s dystrophin genotype would give rise to DCM. Rather, the most relevant question for patients is how soon they will develop DCM based on their own dystrophin mutation, so that early cardiac surveillance and preventive measures can begin in a timely manner to potentially delay DCM and improve quality of life. The investigation of how dystrophin genotype affects each patient’s age of DCM development from the perspective of understanding how genotype modifies the protein’s molecular conformation and cellular function has not been pursued.

Study Purpose

To determine age of DCM manifestation in relation to dystrophin genotype and its resulting dystrophin protein modifications in BMD and XLDCM patients.
Specific Aims

1. To determine the association between DCM age of onset and dystrophin deletion mutations when patients are categorized based on the affected dystrophin protein domain boundaries. The hypothesis is that the loss or alteration of specific functional domain(s) of the dystrophin protein will be associated with earlier age of DCM manifestation.

2. To determine if structural arrangements of the dystrophin rod-domain spectrin-like repeats affect age of DCM manifestation among BMD patients. The hypothesis is that severe disruption of dystrophin protein structure will lead to younger age of DCM manifestation.

Theoretical Framework

The theoretical foundation (Figure 8) that guides the logic of the design and analysis of this dissertation study was extracted from two seminal human and animal studies (Harper et al., 2002; Monaco et al., 1988). The three constructs, genotype, protein structure and phenotype are the main stance of the model, and they are color-coordinated with the concepts (Figure 8). Concepts in green background originate from the reading-frame theory proposed by Monaco et al. (1988) and guide the process of subject selection. Concepts in orange background are established based on findings of Harper et al. (2002). They guide the data analysis approach to establish a genotype-phenotype correlation. Table 2 is a detailed definition and description of each construct and concept in the theoretical model.
The basis of the theory stems from the understanding that DNA encodes RNA, which then encodes protein through the transcription and translation processes. Acknowledging epigenetic mechanisms, the correct DNA sequence is a primary determinant of the correct coding for the protein, which is the functional unit of normal physiology. Monaco and colleagues (1988) first observed that intragenic deletions that result in shifting of the subsequent triplet codon reading frames (frame-shift) were primarily carried by DMD patients, whereas deletions that result in in-frame mutations were mostly found in BMD. As a result, a “reading frame theory” was proposed by Monaco (1988), contending that severe deficiency or truncation of dystrophin protein found in DMD is due to the loss of the translational reading frame, whereas the dysfunctional or partial dystrophin protein found in BMD is due to in-frame mutations. Later, Koenig et al (1989) conducted a genotype-phenotype analysis on 258 DMD and BMD patients to test Monaco’s (1988) reading frame theory. The genotype-phenotype linkage found in 92% of the subject in this study was supportive of the theory.

In the animal study by Harper and colleagues (2002), different dystrophin constructs carried by the transgenic animals were composed based on varying assemblies of dystrophin’s spectrin-like repeats. Functional and molecular analyses of these transgenic animals demonstrated that the phasing pattern of spectrin-like repeats affected dystrophin function. In this experiment, one transgenic mouse expressed dystrophin exon deletions resulting in the coding of 8 full spectrin-like repeats, forming an “in-phase” composition of the central rod domain. This group of animals was compared with another group from an earlier study (Phelps et al., 1995), which had dystrophin exons 17-48 deletion, forming 8 full and 1 partial (out-of-phase) spectrin-like repeats in the rod
domain. As shown in Figure 9, animals with dystrophin deletion of exons 17-48 partially expressed spectrin-like repeat 19, whereas another group did not express spectrin-like repeat 19 at all. Even though dystrophin from the second group of animals had lower molecular weight than the first group (222kD vs. 228kD), their functional and molecular outcomes were close to their wild-type counterparts. These findings indicated that in-phase composition of dystrophin rod domain led to similar quadriceps and diaphragm muscle morphology. They also had similar specific contractile force levels of diaphragm and extensor digitorum longus muscles, compared to wild type animals (Harper et al., 2002; Phelps et al., 1995).

While Monaco et al. (1988) and Harper et al. (2002) tested the reading frame theory and the impact of spectrin-like repeat phasing on the skeletal muscle outcome in human and mouse, no previous effort has explore how the human patient’s cardiac phenotype may be affected by these mechanisms. The reading-frame hypothesis (Monaco et al., 1988) supported that BMD patients are the best population for studying functional roles of specific dystrophin gene regions, due to the fact that they have dystrophin protein in their muscle. By correlating the BMD patient’s clinical muscle performance deficit with their genotype, one can speculate the function of the mutated region of dystrophin. These findings of Harper et al. (2002) established the theoretical hypothesis that for those BMD patients whose dystrophin deletion mutations affected the central rod domain of dystrophin, their clinical outcomes would be worse (e.g. younger age of DCM manifestation) when the mutation is disruptive to the spectrin-like repeats structure (out-of-phase). Likewise, when the BMD patient’s mutation resulted in an in-phase structure of the spectrin-like repeats, they may present with a better outcome as
measured by older age of DCM manifestation. Extending the skeletal muscle findings from Harper et al. (2002) to cardiac studies in BMD patients, a hypothesis is formed based on the impact of the phasing pattern of spectrin-like repeats and is used to guide the analysis of DCM clinical phenotype variations in the BMD population.
CHAPTER 3

RESEARCH DESIGN AND METHODS

General Procedure

Research protocol was approved by the Institutional Review Board (IRB) at Nationwide Children’s Hospital (NCH), which waived additional IRB review by The Ohio State University (OSU) due to contractual agreement between the two institutions. Patients were enrolled from the Muscular Dystrophy Clinics at NCH and the OSU Medical Center (OSUMC). Medical records of BMD patients from NCH and OSUMC were reviewed. For patients who had been clinically diagnosed with BMD but had not been genotyped, the clinical research staff at both clinics invited the patients to consider participation in the national United Dystrophinopathy Project (UDP), which is based in the University of Utah, Salt Lake City, Utah. By agreeing to be a UDP participant, the patient provides a blood sample that is sent to the UDP Center at University of Utah to be genotyped free of charge.

Permission was obtained to access clinical information of patients enrolled in UDP, providing another source for patients for this study. In addition, a comprehensive literature search was conducted to collect published case reports on BMD and XLDCM patients. Search engines utilized included PubMed and Google, in addition to permitted access to the Leiden Open Variation Database (Leiden University, the Netherlands), a
voluntary world registry of dystrophin mutations. Inclusion/exclusion criteria are described below under Data Collection.

All patients were de-identified and assigned a unique identification number. A separate file was kept to track their names and where they were from. A comprehensive database was generated as a Microsoft Excel file to store all patients’ clinical information prior to statistical analyses. Collection of clinical information included the following if available:

- Mutation of DNA (e.g. c.494A>T; in-frame or out-of-frame)
- Mutation of RNA (e.g. Exon 45-48 deleted)
- Mutation of amino acid (i.e. p.2740Asp>Gly)
- Affected exon(s) (e.g. exon 50~51)
- Type of mutation (e.g. deletion)
- Phasing pattern of the dystrophin spectrin-like repeats (e.g. in-phase or out-of-phase)
- Technique used for genotyping
- Clinical diagnosis
- Source (e.g. UDP or Leiden)
- Clinical history in narrative form, including age of skeletal symptoms onset, initial symptoms, age of diagnosis, wheelchair dependency, immunohistochemistry results from muscle biopsies, muscle strength, medications, pulmonary function test results, serum CK level, mental disability/disturbance, family history, date of birth, gender and race.
• Cardiac history in narrative form, including age of first cardiac symptoms (if any), age and detailed results of electrocardiogram, echocardiogram, available cardiac MRI, chest X ray, cardiac catheterization or any other procedural/physical examinations of the heart, history of cardiac surgeries/diseases, and cardiac medications and dosages.

• Assigned cardiac function category (as defined in Table 3).

To ensure the independence of subjects, when siblings occurred in the database, only one from each pair was included in statistical analyses. For statistical analysis, an SPSS (v15, SPSS Inc., Chicago, Illinois) file was generated and some of the above information, particularly age, mutations, echocardiographic results and cardiac function categories, were entered using corresponding number codes (e.g. BMD = 1; XLDCM = 2).

Grouping of Patients

To determine whether genetic mutations affecting specific functional domains of dystrophin protein correlate with an earlier age of cardiac involvement, patients were categorized into 3 groups based on the functional domain affected by the exon deletion (Figure 10). Group 1 included patients with mutations that affected any portion of the amino-terminal actin-binding domain of dystrophin (exons 2 to 8). The region surrounding Hinge 3 was separated into two groups based on prior reports from BMD patients that correlated between skeletal muscle involvement and whether Hinge 3 was affected in the protein product (Carsana et al., 2005). Group 2 included patients with deletions contained within exons 45 to 49. These deletions affect spectrin repeats 17 up
to 19 but leave Hinge 3 intact. Group 3 included patients with deletions affecting exons 50 and/or 51, thus removing or disrupting Hinge 3. These patients had deletions that span from exon 45 up to exon 55 and affect spectrin repeats 17 up to 21.

Dystrophin Protein Modeling

Dr. Will Ray, a collaborating biophysicist of this study, conducted the 3-dimensional modeling of dystrophin rod domain to study how in-phase and out-of-phase deletion mutations would affect the structure and stability of dystrophin. The rod-region spectrin-like repeats were modeled based on the published structure of repeats 15 and 16 of chicken brain alpha spectrin (RCSB Protein Data Bank code 1U5P) (Kusunoki, Minasov, Macdonald, & Mondragon, 2004). The structure was manually extended by replication and root mean square alignment of corresponding terminal amino acid residues, using PyMol (http://www.pymol.org/). The structure was briefly minimized using molecular dynamics within VMD/NAMD (http://www.ks.uiuc.edu/Research/namd/), a biomolecular simulation and analysis program. The minimization was carried out in 4 steps by solvating each manually constructed repeat structure within NAMD, freezing the structure and minimizing the waters to the structure, releasing the waters and freezing the backbone and minimizing side chain rotamers, releasing the backbone and minimizing the entire repeat structure within the frozen water, then finally releasing the entire system and applying 5000 2fs steps of molecular dynamics at 310K (W. C. Ray, personal communication, February 20, 2009). This minimization was not intended to predict the fine-grained structural stability of the various conformations, but eliminated exceptionally bad chain conformations and
steric or electrostatic interferences that may have been created by the manual modeling procedure. The 3-dimensional schematic of dystrophin spectrin-like repeats 16-20 constructed by this method is seen in Figure 11B. The hinge region and out-of-phase deletion mutation structures were constructed by manual deletion of structurally equivalent residues from the extended spectrin repeat, and structural re-alignment of the resulting fragments. A similar minimization was used to finalize the structural compaction resulting from the deletion mutations.

Echocardiography

Echocardiography evaluation is sensitive to anatomical and physiological changes of a dilated heart. Signs of DCM sensitive to direct visualization and measurements in an echocardiography study include: dilated ventricles and atria, areas of dyskinesis and akinesis, increased left ventricular volume, decreased ventricular wall thickness, and mitral regurgitation. With additional calculations, echocardiography is also sensitive to other measurements that can reflect cardiac geometric distortion consequential to DCM, including (1) ejection fraction ([left ventricular diastolic volume – left ventricular systolic volume] / left ventricular diastolic volume); (2) shortening fraction ([left ventricular end-diastolic diameter – left ventricular end-systolic diameter] / left ventricular end-diastolic diameter); (3) E-point septal separation (EPSS, the minimal separation between the mitral valve anterior leaflet and the ventricular septum during early diastole); (4) sphericity index (left ventricular short to long axis ratio); and (5) cardiac mass (Anderson, 2007; Solomon, 2007; Tani et al., 2005).
The following criteria defined presence of cardiomyopathy in this study: ejection fraction below 55%, shortening fraction below 33%, diagnosis of cardiomyopathy, or cardiomegaly. These criteria were determined based on established standards in the DMD and BMD populations (Jefferies et al., 2005; Duboc et al., 2007). When available, additional parameters were included to confirm cardiac dilation: EPSS or left ventricular end diastolic dimension (Table 3.1). An EPSS of greater than 5-5.5mm suggests that the left ventricle has become more dilated and provides a clue for cardiac functional changes (Anderson, 2007). In the adult, the left ventricular internal dimensions during systole and diastole in a normal heart are <37mm and <56mm, respectively (Solomon, 2007). In this study, left ventricular internal dimension >58mm described DCM.

Age

Age of cardiac manifestation was reported as the youngest age at which the patient’s cardiac parameters meet the definition of cardiomyopathy, or the oldest age at which the patient is defined as unaffected with cardiomyopathy as outlined above. The purpose of defining “age” differently for cardiomyopathic versus non-cardiomyopathic patients was to best capture the true age of DCM onset. For example, if a patient had cardiac data showing normal at age 17, and DCM at ages 25, and 28, respectively, the age of 25 will be collected as his age of DCM onset, because this is the age closest to when his DCM was noted. On the other hand, if a patient’s cardiac data showed normal, normal, and normal at ages 33, 36, and 45, respectively, the age collected for this patient would be 45, since this is the age closest to when he actually developed DCM.
Samples

Although DMD, BMD and dystrophin-related XLDCM patients have mutations in their dystrophin gene, DMD patients were excluded. This is because the minimal phenotypic variation in DMD patients caused by dystrophin mutations is primarily frame-shifting and it uniformly gives rise to no dystrophin protein expression and severe muscle degeneration. A meaningful genotype-phenotype association cannot be established when there is a variety of genotypes yet only one phenotype (lack of dystrophin protein in the case of DMD). For this reason, only those patients with confirmed dystrophinopathy and whose diagnosis was not DMD were enrolled in this study. It thus included BMD and dystrophin-related XLDCM.

Data from BMD and XLDCM with dystrophin gene in-frame deletion mutations of less than 12 exons were collected from sources discussed under General Procedure. The reason to only have included patients with exon deletion mutation was because deletion mutation is the most common among BMD patients. Thus, it was realistic to plan to conduct a statistical genotype-phenotype analysis based on a population that was large enough to generate meaningful data. Furthermore, even if non-deletion mutations such as duplications and insertions were included, it would not have been appropriate to mix them with deletion mutations because each type of mutation affects the protein differently. The deletion size limit of less than 12 exons was selected so that we could include enough number of BMD and XLDCM patients to maintain statistical power, while avoiding deletions that are large enough to affect more than one functional domains of the dystrophin protein, which would lead to misinterpretation of the genotype-phenotype correlation.
Power analysis for sample size estimation for single-gene genotype-phenotype correlation studies in dystrophinopathy or other rare disorders has not been proposed. The sample sizes of past studies as well as this dissertation study were limited to the availability of eligible patients. The goal of this study was to recruit more than 100 BMD or XLDCM patients, which would be the largest number to date. New BMD patients at NCH or OSU clinics were referred to Dr. Hugh D. Allen at NCH for cardiac evaluation and echocardiography to minimize inter-rater variability.

Data Collection

To be included in this study, patients must have: (1) a diagnosis of BMD and/or XLDCM; (2) confirmed exon deletion mutations encompassing less than 12 exons; and (3) echocardiographic measurements or compelling clinical evidence for DCM, which combines echocardiographic abnormalities, heart failure, cardiomegaly or cardiac transplantation. Patients were excluded if their dystrophin gene mutations affected the first exon or the 3’ untranslated region, or if their cardiac biopsies showed no dystrophin expression. BMD patients were excluded if they were wheelchair-dependent by 12 years of age, were younger than 12 years of age without family history, had out-of-frame mutations and if no muscle biopsy was available to confirm dystrophin protein expression. Finally, patients with reported or suspected cardiac viral infection were excluded.
Data Analysis

The non-parametric Kruskal-Wallis test (one-way ANOVA by ranks) was performed for cross-group comparisons of age \((a \text{ priori } p < 0.05)\). Mann-Whitney U tests were utilized for post hoc comparisons among groups \((a \text{ priori } p < 0.016\) to achieve overall significance of \(p < 0.05\) using the Bonferroni adjustment). For those with mutations in dystrophin central rod domain, the difference in age of DCM manifestation was used to indicate the consequences of spectrin-like repeat structural changes. This age difference between patients with in-phase and out-of-phase mutations was analyzed with a two-sample \(t\)-test \((a \text{ priori } p < 0.05)\). All data were analyzed in SPSS (v15, SPSS Inc., Chicago, Illinois).
CHAPTER 4

FINDINGS

General Description of Samples

Data from 309 patients were initially collected based on their medical diagnoses of BMD or dystrophin-related XLDCM. After applying inclusion and exclusion criteria, data from 110 patients were included in this study. The age range for patients with DCM was 12 to 76 years, while the age range for patients without DCM was 3 to 50 years. Detailed clinical information, including genotype, musculoskeletal and cardiac involvement information on each patient is presented in Appendices A and B as supplemental data.

In this study, “age” was defined as follows: for those affected with DCM, it was the youngest age at which the patient’s cardiac parameters met the definition for DCM according to criteria listed in Table 3; for those unaffected with DCM, it was the oldest age at which the patient’s cardiac status was considered normal according to the same criteria. Table 4 shows number of patients according to diagnosis and source, as well as median ages based on cardiomyopathy categorization. As shown, the non-cardiomyopathic patients were significantly younger than the cardiomyopathic ones (p < 0.001), regardless of the source. This suggested that the non-cardiomyopathic patients
may be unaffected because they were not old enough to develop heart disease, rather than having specific genotypes protecting them from developing DCM.

To determine whether all cardiomyopathic patients can be combined for maximum statistical power in genotype analysis, we tested whether the clinical diagnosis (e.g. BMD vs. XLDCM) or the source of patient information (e.g. from publications vs. not from publications) would influence age of DCM onset. When comparing the ages of DCM onset between cardiomyopathic patients with either XLDCM or BMD diagnosis, no statistical differences were found. Further, when comparing the median ages for cardiomyopathic patients across data sources, we found no statistically significant differences between patients collected from published literature versus those from NCH and OSUMC. These results demonstrated that it is appropriate to combine all BMD and XLDCM patients for genotype-phenotype analyses even though they may have different clinical diagnoses and were from different sources.

Next, deletion mutations for cardiomyopathic patients (n = 76) were mapped out graphically to determine whether different dystrophin protein domains were affected in XLDCM versus BMD patients (Figure 12). The deletion mutations found in BMD and XLDCM patients clustered around the same two regions of the dystrophin protein: the amino-terminal domain corresponding to exons 2 to 13, and a region in the rod domain centered around Hinge 3, corresponding to exons 45 to 55. This distribution was in agreement with previous reports on mutation hotspots for BMD patients (Worton & Thompson, 1988; Worton, Molnar, Brais, & Karpati, 2001). In addition to the similar mutation distribution, some particular mutations, such as exons 45-48 or 45-55 deletions, were shared by patients with both diagnoses.
Mutation Group 1

Mutations of all cardiomyopathic patients are illustrated in Figure 13 by mutation group. As defined in Chapter 3, patients in Group 1 were those with mutations that affected any portion of the actin-binding N-terminus domain of dystrophin (exons 2 to 8). Ten patients in Group 1 were cardiomyopathic and 4 were non-cardiomyopathic, with median ages 22.5 years and 19.5 years, respectively (Figure 14). Lack of age related statistical significance between these two groups was most likely due to the small sample size. From the age information of Group 1 cardiomyopathic patients, there were 2 sub-groups, one in the mid-teens, and the other in the late 20’s (Appendix A). Further investigation on additional parameters that might provide a hint that there were indeed 2 sub-groups showed that neither the exon deletion pattern nor the onset and severity of skeletal muscle symptoms redistributed Group 1 patients into two distinct sub-groups. Among all 3 groups, Group 1 patients showed the widest spectrum of skeletal muscle involvement, ranging from clinically undetectable muscle weakness to loss of ambulation in the late teens (Appendices A & B).

Mutation Group 2

Group 2 included patients with deletions contained within exons 45 to 49. These deletions affect spectrin repeats 17 up to 19 but leave Hinge 3 intact. The majority (67%) of patients in this study had Group 2 mutations. This group included 54 cardiomyopathic and 20 non-cardiomyopathic patients. The median age for DCM was 29.5 years, significantly older than the non-cardiomyopathic patients (18.5 years) (p < 0.001) (Figure
The age range for DCM was from 15 to 55 years, with the exception of one outlier (ID: 161), who was diagnosed at age 70 with a low ejection fraction of 27%, suggesting advanced disease that had not been detected earlier (Appendix A). Most patients in this group, whether affected with cardiomyopathy or not, showed mild skeletal muscle involvement, with only a few patients who became wheelchair-dependent in their mid 20’s to late 40’s (Appendices A & B). Eight cardiomyopathic patients in Group 2 had cardiac biopsy data which showed detectable cardiac dystrophin, yet it was fainter than control and discontinuous along cardiomyocyte membrane.

**Mutation Group 3**

Group 3 included patients with deletions affecting exons 50 and/or 51, thus disrupting or removing Hinge 3. These patients had deletions that spanned from exon 45 to exon 55, affecting spectrin repeats 17 up to 21. Twelve had DCM and 9 did not. The median age for the cardiomyopathic patients in Group 3 was 46.5 years, the oldest among all three groups. The cardiomyopathic patients were significantly older than their non-cardiomyopathic counterparts, whose median age was 9 years (p < 0.001) (Figure 14). Among cardiomyopathic patients, two older patients with deletion mutations spanning exons 45 to 55 showed only asymptomatic borderline cardiac involvement at ages 69 (ID: 162) and 76 (ID: 248). Even though their ejection fraction values were above 55%, patient 162 showed cardiomegaly by chest roentgenogram and patient 248 had a reduced shortening fraction of 28.9%. Cardiac biopsies with dystrophin immunostaining from 4 cardiomyopathic patients in this group showed detectable dystrophin staining with a discontinuous pattern at reduced levels. It is interesting to note that all patients in Group
Results for Research Aim 1

The first research aim was to determine the association between DCM age of onset and dystrophin deletion mutations when patients are categorized based on the affected dystrophin protein domain boundaries. The median ages of DCM manifestation for patients in each mutation group were calculated as described above. They were 22.5, 29.5 and 46.5 years for Groups 1, 2 and 3 mutations, respectively (Figure 14). The non-parametric Kruskal-Wallis test showed significant differences in age among the groups (p < 0.0005). Significant differences were detected for all post-hoc Mann-Whitney U pairwise comparisons: p = 0.010 for Group 1 versus Group 2; p < 0.001 for Group 1 versus Group 3; and p = 0.001 for Group 2 versus Group 3. Therefore, deletion mutations affecting these three separate domains of the dystrophin protein were associated with significant differences in the age of cardiac manifestations. More specifically, patients with deletion mutations affecting exons 2 to 9 or exons 45 to 49 were at risk of developing DCM in their second and third decade of life, respectively. In contrast, BMD patients with deletions that were within exons 45 to 55 resulting in a dystrophin protein lacking Hinge 3 were protected from early-onset DCM.
Results for Research Aim 2

The second research aim was to determine if structural re-arrangements of the dystrophin rod-domain spectrin-like repeats affect age of DCM manifestation among BMD patients. At the rod domain of dystrophin, 50 exons (exons 10-60) encode for 24 spectrin-like repeats as well as Hinges 1 and 2. The exon boundaries at the gene level do not correspond to the boundaries of each spectrin-like repeat at the protein level (Figure 4). Different exon deletions result in different re-arrangements of spectrin-like repeats (e.g. either in-phase or out-of-phase). As illustrated in Figure 15, several possible exon deletion patterns, such as deletions of exons 46-49, 48-49, 45-46, 45-48 and 47-48, will all predict the in-phase composition of the spectrin-like repeats. Likewise, a number of exon deletions, such as deletion of exons 45-47, will disrupt the structure of the spectrin-like repeats and thus make them out-of-phase.

Transgenic animals over-expressing dystrophin with an in-phase rod domain demonstrate a better phenotype as compared to those over-expressing an out-of-phase rod domain (Harper et al., 2002). With a large number of DCM-affected patients in mutation Group 2 (n = 54), we were able to examine this research aim statistically. For Group 2 mutations, there were 10 unique types of exon deletion mutations. Each of these mutations and its resulting spectrin-like repeats phasing are listed in Table 5. Evaluation of the age of patients in Group 2 based on their spectrin-like repeat phasing showed that patients with in-phase mutations had a significantly later age of DCM manifestation (36.5 years) compared to those with out-of-phase mutations, (25.5 years) (p = 0.002). These data are shown in Figure 16.
We then compared the age of DCM manifestation of Group 2 mutations (both in-phase and out-of-phase) individually with Group 1 and Group 3 mutations (Table 6). The age of DCM manifestation for Group 2 in-phase mutations was significantly older than Group 1 mutations (36.5 vs. 22.5 years, p < 0.001) but did not differ from that of Group 3 mutations. The age of DCM manifestation for Group 2 out-of-phase mutations did not differ from that of Group 1 mutations but was significantly younger than Group 3 mutations (25.5 vs. 46.5 years, p < 0.001). Thus, out-of-phase mutations in the rod domain and deletions in the amino-terminal actin-binding domain are both associated with early age cardiomyopathy. These results indicated that the effect of deletion mutations on the phasing pattern of spectrin repeats 17 to 19 strongly correlates with the age of onset of cardiomyopathy.

We explored whether the earlier onset of DCM observed in out-of-phase mutations was due to absence of cardiac dystrophin protein expression. Based on available cardiac biopsy information (Appendix A), the expression of a mutant cardiac dystrophin protein was present in both in-phase and out-of-phase mutations (Arbustini et al., 2000; Fanin, Melacini, Angelini, & Danieli, 1999; Maeda et al., 1995; Muntoni et al., 1997; Politano et al., 1999). This suggests that the significant difference in the age of DCM onset between in-phase and out-of-phase mutations is not due to obvious differences in the level of cardiac dystrophin expression.

Collaborating with Dr. Will Ray, the predicted models of dystrophin rod domain as a result of either in-phase or out-of-phase mutations were established using methods described in Chapter 3. In-phase mutations were predicted to have the effect of shortening the rod domain (Figure 17A), whereas out-of-phase mutations were predicted
to graft non-homologous sub-regions together (e.g. Helix 1 onto Helix 2 and vice versa), leading to much more detrimental changes to the rod domain structure (Figure 17B and C). Such major alterations to the overall protein structure are likely to affect the function of dystrophin. Utilizing the mutation of exons 45-47 deletion as the example for the out-of-phase structure, we showed that this particular mutation caused an attachment of a Helix 2 onto the adjacent Helix 1 (asterisk, Figure 17B), losing a flexible linker sequence. This structural alteration reverses the direction of extension of the Helix 1 and defines a new axis for the latter portion of the rod. Most other out-of-phase mutations were predicted to bend the rod and reverse its direction, with the exception of exon 48 deletion, which was predicted to give rise to the creation of a new hinge, geographically close to Hinge 3 (Figure 17C).

Additional Findings

Rare Deletion Mutations

Five patients meeting inclusion/exclusion criteria were eventually removed from this genotype-phenotype analysis. These patients had deletion mutations between exons 10 and 20. These patients were affected with DCM and had deletion mutations of exons 10-16 (n=1), 11-13 (n=1), 13 (n=2) and 13-19 (n=1), and their median age of DCM manifestation was 40.6 years old. Elimination of these patients does not imply non-significance of the mutations. Rather, the number of patients in this group was too small to be analyzed statistically.
In the process of data collection, we encountered several cases of dystrophin point mutations. The genotype-phenotype association in these patients was not analyzed due to the rarity of dystrophin point mutations. As shown in Figure 18, 8 patients with dystrophin point mutations had the XLDCM clinical diagnosis. These patients were identified in published literature, with the following mutations: (1) 18Lysine>asparagine (n = 1) (Feng et al., 2002); (2) 279threonine>alanine (n = 5, all from same family) (Berko & Swift, 1987; Ortiz-Lopez, Li, Su, Goytia, & Towbin, 1997); (3) 1672 asparagine>lysine (n = 1) (Feng, Yan, Buzin, Sommer, & Towbin, 2002); and (4) 3228 phenylalanine>leucine (n = 1) (Feng et al., 2002). The phenotypic presentation of all of these patients was cardiac-specific without any skeletal muscle symptoms. The DCM in these patients was very severe, with the average age of cardiac symptom manifestations being 17 years old. In particular, a previous study revealed that the amino acid substitution of threonine to alanine at position 279 (Hinge 1) disrupts the sequence of a evolutionarily highly conserved region, leading to a change in amino acid polarity and consequently substituting a β-sheet for α-helix and changing the secondary and tertiary protein structure of dystrophin (Kahana, Marsh, Henry, Way, & Gratzer, 1994; Koenig & Kunkel, 1990; Ortiz-Lopez et al., 1997).

It is interesting that all of these point mutations occurred at regions where dystrophin is known to have interactions with its neighboring proteins, such as actin and the dystroglycan complex. Further, from the conservation analyses performed by our collaborating geneticist, Dr. Carlos Alvarez, we found that all of these loci are highly evolutionary conserved. Data from conservation analyses are shown in Figures 19 to 22.
These two mechanisms may explain the severe and early-onset DCM seen in these patients.

In addition to XLDCM patients with dystrophin point mutations, 3 BMD patients also had point mutations. Their mutations were: (1) 165aspartic acid>valine (at N-terminus); (2) 2740aspartic acid>glycine (rod domain at exon 56); and (3) 3368aspartic acid>glycine (at C-terminus). All 3 patients were from the UDP patient pool. The patients with 165aspartic acid>valine and 3368aspartic acid>glycine mutations were affected with DCM at 17 and 46 years old, respectively. The patient with 2740aspartic acid>glycine mutation was unaffected with DCM at 34 years of age.
CHAPTER 5

DISCUSSION AND CONCLUSION

Study Summary

The primary strengths of this study were the large sample size (N=110) and the stringent selection criteria that only included patients with small deletions, allowing the grouping of patients based on mutations affecting well defined regions of the dystrophin protein without compromising statistical power. Due to the low incidence of BMD, previous genotype-phenotype correlation studies as reviewed in Chapter 2 were too underpowered to detect the effects of mutations in regions of the dystrophin gene that are not mutation hot-spots. The number of patients analyzed in past studies ranged from 19 to 84 and included patients with very large exon deletions (up to 43 consecutive exons).

In this dissertation, we studied patients with dystrophin exon deletion mutations collectively without separating them by clinical diagnoses, since high genetic (e.g. mutation distribution) and phenotypic (e.g. DCM age of onset) similarities between BMD and XLDCM patients were found. Median ages of cardiac manifestation were identified with respect to 3 distinct regions of dystrophin protein as well as to specific patterns of the rod-domain spectrin-like repeat re-arrangements. The earliest age of DCM development, 22.5 years, was associated with dystrophin deletion mutations affecting exons 2 to 9 (Group 1). In comparison, deletion mutations in the region between exons
45 and 55 which resulted in removal of Hinge 3 of the dystrophin protein (Group 3) were found to be associated with the latest onset of DCM, 46.5 years. Giving rise to DCM age of onset in-between the youngest and the oldest ages were deletion mutations affecting exons 45 to 49 (Group 2). The median age of DCM onset for this patient group was 29.5 years. Interestingly, when this group was divided into 2 subgroups based on the phasing pattern of the affected spectrin-like repeats, we found that out-of-phase deletion mutations led to an earlier age of DCM onset, 25.5 years, compared to 36.5 years for in-phase deletion mutations. Further, 3-dimensional modeling studies suggested that the earlier DCM age of onset may be explained by the predicted aberrant grafting of non-homologous sub-regions of dystrophin rod-domain spectrin-like repeats, causing severe changes to the protein’s structure and possibly impacting function.

Discussion

Exon Deletions in the Amino-Terminal Domain

Deletions affecting the 5’ region of the dystrophin gene, including the muscle promoter region, exon 1, or intronic regions that alter exon splicing, have been associated with a mild clinical course of skeletal muscle degeneration yet with severe DCM. For these patients, the preferential cardiac involvement was found to correlate with the complete absence of cardiac dystrophin. The molecular mechanisms underlying this phenomenon include tissue-specific use of alternative dystrophin promoters, disruption of tissue-specific enhancers, and differences in alternative splicing between skeletal and cardiac muscle (Muntoni et al., 1994; Kimura et al. 2007; Bies et al., 1997; Milasin et al. 1996; Muntoni et al., 1993; Muntoni et al., 1995; Neri et al., 2007). In order to minimize
the error of misinterpreting that the patient’s age of DCM onset was due to his dystrophin genotype rather than the absence of cardiac dystrophin protein, we purposefully excluded those known to lack cardiac dystrophin expression in this study. Because of the finding that the age of DCM development in XLDCM patients with no cardiac dystrophin reported in the literature was very similar to that of our patients in Group 1, we speculate that despite our effort to eliminate patients known to have no cardiac dystrophin, at least some of our Group 1 patients still owe their early DCM manifestation to the lack of cardiac dystrophin. Nevertheless, given the variety of different exon deletion patterns in Group 1, the probability for the majority of Group 1 patients to have early DCM because of this particular cause is small.

An alternative hypothesis to explain the early-onset DCM and milder musculoskeletal symptoms in patients with mutations in the amino-terminal domain is that although dystrophin’s affinity for actin is lost in both cardiac and skeletal muscle resulting from the deletion, the effect may be more severe in the cardiac muscle due to its different mechanical stress, geometry and physiology. Several lines of evidence support this possibility. One study showed that approximately 35% of the cardiac dystrophin protein tightly binds to actin and is an important part of the cardiomyocyte contractile apparatus. In the presence of cardiomyopathy, this association is progressively disrupted as demonstrated in hamster models (Meng, Leddy, Frank, Holland, & Tuana, 1996). More recent studies have also confirmed that alterations in cardiac dystrophin, specifically cleavage of the amino-terminal domain, accompany and may precede cardiac dysfunction not related to dystrophin mutations in both rodents and humans (Vatta et al., 2002; Toyo-Oka et al., 2004). Taken together, these observations suggest that in the
cardiac muscle, the amino-terminal domain of dystrophin plays an essential role in tethering and stabilizing the contractile apparatus via its binding with actin and that loss of this link leads to cardiomyopathy.

*Exon Deletions in the Rod Domain*

The rod domain of dystrophin is usually considered as a spacer with elastic properties (Ervasti & Sonnemann, 2008). Since mutations giving rise to a dystrophin protein with either a longer or shorter rod domain are typically associated with a late onset of skeletal muscle symptoms and a slow disease progression, dystrophin rod domain appears to be permissive to alterations without much major or severe impact on skeletal muscle function (England et al., 1990; Dastur, Gaitonde, Khadilkar, & Nadkarni, 2008; Mirabella et al., 1998). This phenomenon has led recent gene therapy researchers to design miniature dystrophin constructs small enough to be packaged into appropriate viral vectors by removing a large portion of dystrophin rod domain (Scott et al. 2002; Draviam, Wang, Li, Xiao, & Watkins, 2006; Bostick et al., 2009).

However, as reviewed in Chapter 2, not all re-arrangements of the dystrophin rod domain are compatible with normal or near-normal skeletal muscle function. Deletions affecting exons 45 to 48 have been associated with the loss of dystrophin’s ability to bind nNOS in humans and mice (Wells et al., 2003). Further, past efforts attempting to substitute dystrophin spectrin-like repeats with homologous spectrin-like repeats from the alpha-actinin 2 protein found that this type of substitution leads to a loss of dystrophin function as well as the development of myopathic skeletal muscle features (Harper et al., 2002). These observations strongly suggest that the rod domain of dystrophin is not just
a spacer; rather, it is likely an important mechanical modulator between the distal dystrophin domains and other proteins and thus is more sensitive to structural alterations than previously thought.

Extending the discussion from skeletal muscle dystrophin to cardiac dystrophin, we questioned whether all rod-domain deletion mutations would have the same impact on the function of cardiac dystrophin. Previous reports have shown that deletions of exons 45-52 and 45-55, although both affecting the distal rod-domain, were associated with normal cardiac function (former) versus congestive heart failure (latter) at relatively similar ages (29 and 36 years, respectively) (Melacini et al., 1996; Tasaki et al., 2001). The phenotypic difference resulting from different rod-domain deletion mutations, as defined by DCM age of onset, was also reflected in our results, particularly between patients whose deletions removed Hinge 3 with those whose deletions preserved Hinge 3.

First, removal of Hinge 3 in Group 3 patients conferred a protective effect in cardiac decline, leading to a significantly later onset of cardiomyopathy. Although prior human and animal studies have shown a correlation between the absence of Hinge 3 and milder skeletal muscle pathology (Ferreiro et al., 2009; Carsana et al., 2005; Harper et al., 2002), this dissertation study is the first to demonstrate that removal of dystrophin Hinge 3 due to exon deletion mutations leads patients to have the oldest age of DCM manifestation (46.5 years old). This was not detected in prior studies because patients with or without Hinge 3 were not analyzed as separate groups (Melacini et al., 1993; Nigro et al., 1994; Politano et al., 1991). Due to the paucity of information on the structure of Hinge 3 and a low number of patients with similar deletions, we could not model the effects of mutations on the dystrophin protein backbone for Group 3 mutations.
Therefore, further studies are needed to elucidate the significant cardio-protective effect conferred by the loss of Hinge 3.

Second, we have shown that deletion mutations preserving proper phasing of the spectrin-like repeats upstream to Hinge 3, specifically the region encoded by exons 45 to 49, significantly delayed DCM development by a decade when compared with out-of-phase re-arrangements in the same region. This is likely not due to difference in cardiac dystrophin protein expression since similar amount and distribution of dystrophin in cardiac biopsy samples from BMD patients with either in-phase or out-of-phase mutations has been reported (Arbustini et al., 2000). Our 3-dimensional modeling of dystrophin rod domain conformation modeled after in-phase and out-of-phase exon deletions suggested that the structural alterations caused by out-of-phase mutations extend beyond the disruption within each spectrin-like repeat unit and lead to a severely distorted rod-domain configuration, ultimately damaging the entire dystrophin protein. This major change in the structure of dystrophin is likely the primary determinant of the early onset of DCM in patients with out-of-phase mutations.

Another interesting finding was that most patients in Group 2 had late-onset skeletal muscle symptoms and mild disease progression, irrespective of the spectrin-like phasing pattern, providing a hint that cardiac dystrophin may be more sensitive than skeletal muscle dystrophin to structural disruption in the exons 45-49 region. Recent studies have tested the ability of synthetic dystrophin constructs with a shortened rod domain to restore cardiac function in the \textit{mdx} mouse. While these constructs rescued skeletal muscle, they were not able to reverse all cardiac parameters to normal (Bostick et al., 2009; Gregorevic et al., 2006; Townsend et al., 2007). Since transgenic constructs
are not subject to alternative splicing or tissue specific differences in exon skipping, these results indicate a genuine difference in dystrophin protein domains required for cardiac and skeletal muscle function.

**Study Limitations**

The genetic, cardiac and other relevant clinical information for most patients in this study was collected retrospectively from published case reports, OSU, NCH and UDP. Most patients had had echocardiography performed by various cardiologists and had been genotyped at different institutions prior to the initiation of the study. To a certain extent, some inter-observer variability was inherent in this research design since taking measurements and making interpretations of an echocardiogram can be subjective and genotyping methodologies vary. Within our ability, we referred newly-identified patients to Dr. Hugh D. Allen at NCH for echocardiographic evaluation and enrolled them in the UDP study for dystrophin genotyping to take place at the UDP headquarters in University of Utah. Although some subjects in this study had echocardiography performed incidentally (e.g. during an emergency room visit), in most cases their echocardiographic evaluations were performed as a protocol of a research program, a setting under which diagnostic procedures are presumed to be of high quality. The genotyping method for the majority of patients was performed with multiplex PCR and Southern blotting techniques, which have low error rates (Prior & Bridgeman, 2005). Other genotyping methods included multiplex ligation-dependent probe amplification (Schwartz & Duno, 2004), detection of virtually all mutations- single-strand conformation polymorphism analysis (Mendell et al., 2001), and single condition
amplification/internal primer sequencing (Flanigan et al., 2003), which have all showed high efficiency and accuracy in detecting small deletion mutations.

Another limitation for this study is the cross-sectional data collection. Due to the rare incidence of BMD and XLDCM, it was not realistic for this study to set out to longitudinally perform cardiac surveillance for a large number of patients prior to cardiac pathology. Therefore, to capture the age closest to the true onset of DCM, “age” for those affected with DCM was defined as the youngest age at which the patient’s cardiac parameters met the definition for cardiomyopathy according to our criteria, and for those unaffected with DCM, “age” was the oldest age at which the patient’s cardiac status was considered normal according to the same standards. We believe that the median age of cardiac involvement for each of the patient groups reported in this study is useful in revealing differences among these groups and is the best approximation available for this patient population.

Nursing Implications

DCM development is part of the BMD disease course. Due to lack of evidence, clinicians cannot define the patient’s risk of developing DCM based on the genetic diagnosis. Consequently, diagnosis and treatment of DCM in these populations often does not begin until cardiac symptoms manifest, leading to poor prognosis and premature death. In this study, we performed a genotype-phenotype analysis to identify dystrophin mutations predictive of the timing for DCM development in patients with BMD or XLDCM. The results demonstrated that genotype is a determinant of the age for DCM development in BMD and XLDCM patients and provided novel evidence to support the
association between age of cardiac phenotype and specific structural alterations of the
dystrophin protein rod domain.

This new knowledge enables nurses in genetic counseling and advanced practice
of cardiology or neurology to customize education for patients and their families on what
to expect with the disease in the context of progressive skeletal muscle degeneration and
cardiac involvement. During the process of data collection, we encountered several
patients who refused to be genotyped because the genotype information would not
change how their disease would be managed (L. Viollet, personal communication, 2008).
The findings of this study may help these patients justify genotyping. In addition, cardiac
function monitoring may be incorporated into routine visits for appropriate patients as a
standard of practice, so that cardiac treatment may be started during early stages of DCM.
For patients with no or mild skeletal muscle symptoms and seem suitable for heart
transplantation, the screening and approval process necessary to place the patient on the
transplant waiting list may be initiated in advance.

The disease natural history of BMD is different from that of DMD because of its
wide spectrum of disease onset and severity that are difficult to foretell. Upon diagnosis
of DMD, most patients and their families can anticipate wheelchair-dependency around
age 12 and the end of life at the third decade. However, BMD patients cannot determine
their disease course with as much certainty. Particularly, knowing that DCM is the
leading cause of death but not when to expect cardiac deterioration in life can bring a
tremendous amount of frustration and stress to the BMD patient and his family. Even
though each patient’s disease course may have individual differences, the findings in this
study on DCM age of onset based on dystrophin genotype can enable the practitioner to
offer the patient and family a general picture of the patient’s cardiac disease progression and prognosis when consulting with patients and families.

Future Research Directions

One direction for future clinical research is to begin longitudinal cardiac monitoring in a large group of BMD patients with detailed genotype data. This will help refine the true age of DCM onset associated with various dystrophin domains identified in this study. Nursing and medical interventions may consequently be designed to prevent or delay the onset and progression of DCM. Because different dystrophin genotypes result in different cardiac phenotypes, individual genotypes potentially also dictate treatment responses to common medication regimen to treat DCM, such as an angiotensin-converting enzyme inhibitor. An important clinical investigation for patients with different genotypes will be to explore factors that may optimize his treatment outcome, through clinical trials testing various dosages, treatment timings, or different ways to combine multiple classes of drugs and integrate lifestyle modifications (e.g. diet, exercise regimen) with pharmaceutical therapies.

Alongside clinical research, it is imperative for laboratory studies to delineate specific mechanisms by which exon deletion mutations in the amino-terminal region of dystrophin lead to such an early onset of DCM when compared with mutations affecting other regions of the protein. Human cardiac tissue samples from explanted hearts may be a valuable source for this research. Alternatively, creating a new animal model of dystrophin-related DCM can also provide a useful in vivo approach to study the genetics and molecular pathology of this disease. Our structural predictions of dystrophin rod-
domain resulting from specific exon deletions as well as how they correlated with different ages of DCM manifestation indicate that normal cardiac function requires structural integrity of the dystrophin protein. This is an important aspect to consider when designing mini- or micro-dystrophin constructs for gene therapy of DCM in this patient population.

Conclusion

The dissertation research was the first to demonstrate that dystrophin genotype is a determinant for DCM age of onset in patients with BMD and XLDCM. Our novel genotype analysis approach highlighted the importance to understand how the protein structure is affected by the genotype when studying genotype-phenotype associations. Our results identified specific dystrophin protein regions that when lost or altered due to gene mutations predispose patients to early- versus late-onset cardiomyopathy. Using this knowledge, nurses in advanced practice and/or in genetic counseling may begin early cardiac surveillance, make timely referrals and facilitate in life-planning for at-risk patients.
APPENDIX A

CLINICAL DESCRIPTION OF PATIENTS IN THIS STUDY AFFECTED WITH DCM
<table>
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<tr>
<th>ID</th>
<th>Deleted Exons</th>
<th>Dx/Age*</th>
<th>Source</th>
<th>Clinical information</th>
<th>Cardiac information</th>
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<tbody>
<tr>
<td>3</td>
<td>2-7</td>
<td>XLDCM, BMD/28</td>
<td>[1] (Patient 1)</td>
<td>Age at consultation: 33 years. Mild myopathy with preferential cardiac involvement beginning from the second decade of life. CK = 800 U/L. Biopsy showed increased central nucleation, fiber splitting and fiber size variation, carnitine deficiency. Slightly fainter dystrophin staining intensity than normal controls. Western blot: reduced dystrophin size. Brother of #251. Negative family history.</td>
<td>Severe congestive cardiomyopathy. EF = 25%.</td>
</tr>
</tbody>
</table>
Mild myopathy with preferential cardiac involvement beginning from the second decade of life. CK = 2000 U/L. Biopsy showed mild myopathy, increased numbers of central nuclei and fiber splitting, fiber size variation. Slightly fainter dystrophin staining intensity than normal controls. Western blot: reduced dystrophin size. Brother of #3. Negative family history.

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<th>ID</th>
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<tr>
<td>SL1032</td>
<td>3</td>
<td>BMD/23</td>
<td>UDP</td>
<td>Onset of skeletal muscle symptoms: 2 years. Age at diagnosis: 5.5 years. Toe walking.</td>
<td>EF = 35%. Palpitations. Peripheral edema. Clinically significant arrhythmia. Heart transplant</td>
</tr>
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<tr>
<td>MJ03</td>
<td>3</td>
<td>BMD/13</td>
<td>MDC</td>
<td>Age at diagnosis: 8 years. Calf hypertrophy. CK = 18,000 U/L. Ambulant at age 14. Negative family history.</td>
<td>Cardiac data at 13 years of age: EF = 50%. Cardiac data at 16 years of age: SF = 28.39%, LV diastolic septal thickness: 0.80cm, LVIDd = 55.3mm (1.02 z-score), LV diastolic wall thickness: 0.80cm. No global LV dysfunction, overall LV systolic wall motion is low-normal. Cardiac data at 18 years of age: LVID = 1.2sd, EPSS = 14mm, SF = 23%, EF = 45%.</td>
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<tr>
<td>MJ18</td>
<td>3-7</td>
<td>BMD/29</td>
<td>MDC</td>
<td>Age at consultation and diagnosis: 25 years. Diffuse muscle stiffness, cramping, weakness. CK = 1200~2000 U/L. Biopsy showed mild muscle fiber atrophy with mild focal chronic inflammation.</td>
<td>Normal ECG. All valves normal, LV global systolic dysfunction and hypokinesis, EF = 35%.</td>
</tr>
<tr>
<td>SL1077</td>
<td>3-11</td>
<td>BMD/19</td>
<td>UDP</td>
<td>Symptom of weakness. Wheelchair bound: 17 years. Vignos Scale: NSI/NSI. Negative family history.</td>
<td>EF = 17%, SF = 8%.</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>XLD/12</td>
<td>[2] (Patient DCM 10)</td>
<td>Age at consultation: 12 years. Presented to the emergency room with dyspnoea, diaphoresis and vomiting for one week. Neurological</td>
<td>DCM diagnosis. LVEDd = 58mm (Z score 5.2), SF = 16%, EF = 30%. Moderate mitral regurgitation.</td>
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exam showed no skeletal myopathy or abnormalities. CK = 270 U/L. Negative family history.

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No coronary artery abnormalities per angiography. Patient died 1 month later. 

**Cardiac biopsy:** non-specific changes with interstitial fibrosis compatible with DCM.

### Group 2 Patients.

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<th>ID</th>
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<tr>
<td>250</td>
<td>45</td>
<td>BMD/31</td>
<td>[5] (Patient 2)</td>
<td>Age at consultation: 31 years. Mild progressive limb-girdle weakness, calf pseudohypertrophy, elevated CK. Biopsy showed discontinuous dystrophin staining.</td>
<td>LVEDd = 58mm, LVEDs = 47mm, EF = 47%, LV posterior wall = 10mm, Interventricular septum = 12mm. <strong>Cardiac Biopsy:</strong> No interstitial fibrosis. Dystrophin staining: discontinuous (partial or intermittent surface membrane staining).</td>
</tr>
<tr>
<td>45</td>
<td>45</td>
<td>BMD/26</td>
<td>[6] (Patient 6)</td>
<td>Age at consultation: 26 years. Major impairment of motor function. Wheelchair bound. FVC = 42%.</td>
<td>ECG showed tall R wave in V1, lateral Q and T abnormal. QTc: 441ms. QT/PQ = 8.6. LVEDd = 66mm. SF = 13%.</td>
</tr>
<tr>
<td>193</td>
<td>45-47</td>
<td>BMD/22</td>
<td>[7] (Patient 1)</td>
<td>CK=265 U/L. Negative family history.</td>
<td>Onset of congestive heart failure symptoms. EF = 25%. <strong>Cardiac Biopsy:</strong> dystrophin staining</td>
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showed reduced with rod domain antibody, irregular with amino- and carboxyl-terminal antibodies.

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<th>ID</th>
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<tr>
<td>253</td>
<td>45-47</td>
<td>BMD/37</td>
<td>[8]</td>
<td>Age at consultation: 37 years. Muscle weakness present.</td>
<td>LVEDd = 58mm, EF = 42%.</td>
</tr>
<tr>
<td>110</td>
<td>45-47</td>
<td>BMD/20</td>
<td>[9] (Patient 20)</td>
<td>Age at consultation: 20 years. Clinical Severity: Mild. Dystrophin staining showed small number of negative fibers. Western blot: reduced amount (40%) and size (360 kDa). Positive family history.</td>
<td>Normal ECG. Normal Holter ECG. Normal LA volume. LV EDV = 64 mL/m². EF = 53%. LV wall motion: normal. RV EDV = 57 mL/m². RVEF = 70%. Normal RV wall motion.</td>
</tr>
<tr>
<td>189</td>
<td>45-47</td>
<td>BMD/35</td>
<td>[10] (Patient B27)</td>
<td>Age at consultation: 35 years. Cramps, myalgia, myoglobinuria, calf hypertrophy. CK = 378 U/L. Western blot: Normal dystrophin amount but reduced size (380 kDa).</td>
<td>ECG showed LBBB. Holter ECG showed polymorphic ventricular arrhythmias (Lown grade 3). LVEDV = 85 mL/m². EF = 39%. LV wall motion: anterior septum akinesia.</td>
</tr>
<tr>
<td>141</td>
<td>45-47</td>
<td>BMD/17</td>
<td>[6] (Patient 1)</td>
<td>Age at consultation: 17 years. Minor impairment of motor function. FVC = 96%.</td>
<td>ECG showed incomplete RBBB, QTc: 399ms. QT/PQ = 10.7. LVEDd = 51mm. SF = 24%.</td>
</tr>
<tr>
<td>ID</td>
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<tr>
<td>146</td>
<td>45-47</td>
<td>BMD/16</td>
<td>[6] (Patient 8)</td>
<td>Age at consultation: 16 years. Minor impairment of motor function.</td>
<td>ECG showed tall R wave in V1, lateral Q and R loss. QTc: 432ms. QT/PQ = 7.36. LVEDd = 48mm. SF = 26%.</td>
</tr>
</tbody>
</table>
*Cardiac Biopsy:* dystrophin staining showed reduced with rod domain antibody, irregular with amino- and carboxyl-terminal antibodies. |
| 192 | 45-47         | BMD, XLDCM/29 | [12] | Age at consultation: 32                                                                                                                                                                                              | DCM diagnosis. ECG showed                                                                                                                                 |

impairment of motor function. FVC = 98%.
Neurological examination: normal except for slight calf hypertrophy. Biopsy showed fiber size variation with scattered atrophic fibers and fiber hypertrophy, internalized nuclei, some necrotic fibers and fiber splitting, moderate increase of connective tissue. Western Blot: slight reduction in dystrophin size.

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<tbody>
<tr>
<td>MJ02</td>
<td>45-47</td>
<td>BMD/34</td>
<td>MDC</td>
<td>EMG at 7 years was normal but calf hypertrophy present. Onset of skeletal muscle symptoms: 22 years. Diagnosed at 32 years. Calf hypertrophy, progressive weakness and heaviness when climbing stairs. CK=1200U/L. Positive family history.</td>
<td>EF = 30%. Family: Grandfather died of DCM.</td>
</tr>
<tr>
<td>MJ20</td>
<td>45-47</td>
<td>BMD/19</td>
<td>MDC</td>
<td>Onset of skeletal muscle symptoms: 10 years. Diagnosed at 11 years.</td>
<td>Cardiac data at 19 years of age: DCM diagnosis. Cardiac medications started.</td>
</tr>
</tbody>
</table>
Symptom of weakness. CK = 1245 U/L. Started cane use at age 19 and wheelchair at 23. Partially ambulatory. Biopsy showed marked variability in muscle fiber size, 10-120 μm. Positive family history.

Cardiac data at 35 years of age: EF = 55%.

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<tr>
<td>AH05</td>
<td>45-47</td>
<td>BMD/39</td>
<td>MDC</td>
<td>Onset of skeletal muscle symptoms: 5 years. Could not keep up with peers in terms of running. Diagnosis: 18 years. Difficulty climbing stairs and getting up from the floor, calf hypertrophy. CK = 2516 U/L. Biopsy showed fiber size variation (10-125 micrometers in diameter), marked connective tissue proliferation. Wheelchair bound by 42 years. Negative family history.</td>
<td>Normal ECG. EF = 51%. SF = 32%.</td>
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<tr>
<td>228</td>
<td>45-47</td>
<td>BMD/37</td>
<td>[13]</td>
<td>Unavailable.</td>
<td>EF = 42%, LVEDd = 58mm. Diagnosis of DCM based on WHO criteria. Negative for cardiac insufficiency based on European Society of Cardiology</td>
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<td>ID</td>
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<tr>
<td>210</td>
<td>45-48</td>
<td>XLDCM/33</td>
<td>[14] (Patient 2)</td>
<td>Age at consultation: 33 years. No skeletal muscle symptoms. CK = 754U/L. Positive family history.</td>
<td>DCM diagnosis predating cardiac data below. Cardiac data at 33 years of age: ECG showed LBBB. Interventricular septal thickness = 8mm; Posterior wall thickness = 8mm; LVEDd = 67mm; LVEDs = 52mm; SF = 22%.</td>
</tr>
<tr>
<td>212</td>
<td>45-48</td>
<td>XLDCM/57</td>
<td>[14] (Patient 4)</td>
<td>Age at consultation: 57 years. No skeletal muscle symptoms. CK = 438U/L. Positive family history.</td>
<td>DCM diagnosis predating cardiac data below. Cardiac data at 57 years of age: ECG showed RBBB. Interventricular septal thickness = 9mm; Posterior wall thickness = 9mm; LVEDd = 65mm; LVEDs = 58mm; SF = 11%.</td>
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<tr>
<td>201</td>
<td>45-48</td>
<td>BMD/33</td>
<td>[15]</td>
<td>Onset of skeletal muscle symptoms: 3 years. Age at consultation: 33 years.</td>
<td>SF = 11%; LVEDd = 82; Interventricular septal thickness = 18mm; Posterior wall thickness: 18.</td>
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<tr>
<td>252</td>
<td>45-48</td>
<td>BMD/54</td>
<td>[8]</td>
<td>Age at consultation: 54 years.</td>
<td>LVEDd = 67mm, EF = 30%, met DCM criteria of WHO. Symptoms: dyspnoea, thoracic pain.</td>
</tr>
<tr>
<td>109</td>
<td>45-48</td>
<td>BMD/15</td>
<td>[9] (Patient 13)</td>
<td>Age at consultation: 15 years. Clinical Severity: mild. Biopsy showed few dystrophin negative fibers. Western blot: reduced dystrophin expression (40%) and size (370 kDa). Negative family history.</td>
<td>ECG showed incomplete RBBB. Normal Holter ECG. LA volume = 40 mL/m². LV EDV = 69 mL/m². EF = 51%. RVEDV = 84 mL/m². RVEF = 63%. RV wall motion apical hypokinesia.</td>
</tr>
<tr>
<td>112</td>
<td>45-48</td>
<td>BMD/30</td>
<td>[9] (Patient 25)</td>
<td>Age at consultation: 30 years. Clinical Severity: Moderate. Biopsy showed small number of dystrophin negative fibers. Western Blot: reduced amount (40%) and size (370 kDa). Positive family history.</td>
<td>ECG showed incomplete RBBB. Holter ECG showed isolated monomorphic PVCs (Lown grade 1). LA volume = 29 mL/m². LV EDV = 78 mL/m². EF = 48%. LV wall motion: diffuse hypokinesia. RV EDV = 64 mL/m². RVEF = 39%.</td>
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</table>
Western Blot: normal amount but reduced size (380 kDa). Positive family history. ventricular arrhythmias (Lown grade 3). Positive infra-Hisian block (His-ventricular interval 80ms). Pacemaker implanted. LA volume = 45 mL/m². LV EDV = 85 mL/m². EF = 39%. LV wall motion: anterior septal akinesia. RV EDV = 69 mL/m². RVEF = 65%. RV wall motion: septal akinesia.

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<tr>
<td>182</td>
<td>45-48</td>
<td>BMD/20</td>
<td>[10] (Patient A14)</td>
<td>Age at consultation: 20 years. Cramps, myalgia, myoglobinuria, calf hypertrophy. CK = 28 U/L. Western blot: reduced dystrophin amount (40%) and reduced size (370 kDa).</td>
<td>ECG showed incomplete RBBB. Normal Holter ECG. LVEDV = 69 mL/m². EF = 51%.</td>
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*Cardiac Biopsy:*
dystrophin staining reduced with rod domain antibody, irregular with amino- and carboxyl-terminal antibodies.

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<tr>
<td>161</td>
<td>45-48</td>
<td>BMD, XLDPCM/70</td>
<td>[16] (Patient 1)</td>
<td>Onset of skeletal muscle symptoms: 60 years. Difficulties in tilling the soil with a hoe and climbing hills and stairs. Age at consultation: 73 years. Neurological exam: moderate proximal muscular atrophy and weakness, waddling gait, mild calf hypertrophy, positive Gower's sign. Walks independently. Computed</td>
<td>Diagnosis of congestive heart failure caused by DCM. Symptoms of paroxysmal chest discomfort and dyspnoea. Chest roentgenogram showed cardiomegaly and pulmonary congestion (cardiothoracic ratio = 61%). ECG showed prominent R wave in V1. EF = 27%, SF = 13.5%, positive LV hypokinesia,</td>
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tomography scan: low-density areas in the proximal muscles, especially in the thighs. CK = 681U/L. Positive family history.

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<tr>
<td>164</td>
<td>45-48</td>
<td>BMD, XLDCM/53</td>
<td>[16]</td>
<td>Onset of skeletal muscle symptoms: early 30's. Awkward gait. Started use of cane in his early 40's. Wheelchair bound in his late 40s. Age at consultation: 53 years. Marked proximal muscular atrophy and weakness, no obvious hypertrophy in the calves. CK = 217U/L. Negative family history.</td>
<td>DCM diagnosis. Chest roentgenogram showed cardiomegaly, bilateral pleural effusion and pulmonary congestion. ECG showed prominent Q wave in I, aVL, V5-6. LV hypokinesis, LVEDd = 64mm, LVEDs = 60mm. EF = 16%, SF = 6.3%.</td>
</tr>
<tr>
<td>SL49</td>
<td>45-48</td>
<td>BMD/47 UDP</td>
<td>Onset of skeletal muscle symptoms: 6.5 years. Diagnosed at 17 years. Myalgia, cramping. Vignos Scale: upper = 1 / lower = 2.</td>
<td>Cardiac data at 47 years of age: SF = 28%. Cardiac data at 52 years of age: EF = 50%.</td>
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<tr>
<td>SL8</td>
<td>45-48</td>
<td>BMD/27</td>
<td>UDP</td>
<td>Onset of skeletal muscle symptoms: 2 years. Diagnosis: 23 years. Weakness, hypertrophy, myalgia/cramping, myoglobinuria, cognitive dysfunction. Started wheelchair at 27 years. Vignos Scale: upper = 1 / lower = 5 at 40 years of age.</td>
<td>EF = 29%.</td>
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well preserved muscle. Dystrophin staining: normal with rod domain antibodies, faint with N-terminal antibody, almost normal with few areas of discontinuity with C-terminal antibody. CK = 520U/L. Condition unchanged at 26 years of age. Brother of #225.

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<tr>
<td>215</td>
<td>45-49</td>
<td>BMD/16</td>
<td>[9]</td>
<td>Age at consultation: 16 years. Clinical Severity: mild. FVC = 109%. Western blot: reduced dystrophin expression (50%) and size (390 kDa). Negative family history.</td>
<td>ECG showed incomplete RBBB. Holter ECG showed isolated monomorphic PVCs documented &gt;30/hr (Lown grade 1). LA volume normal. LV EDV= 65 mL/m². EF = 50%. RV EDV = 45 mL/m². RVEF = 51%.</td>
</tr>
<tr>
<td>SL48</td>
<td>45-49</td>
<td>BMD/28</td>
<td>UDP</td>
<td>Onset of skeletal muscle symptoms: 6.5 years. Diagnosis: 6 years.</td>
<td>EF = 40%.</td>
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Calf hypertrophy, myalgia, cramping. Started wheelchair at 22.5 years. Vignos Scale: upper = 1 / lower = 2.

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<tr>
<td>147</td>
<td>46-49</td>
<td>BMD/26</td>
<td>[6] (Patient 11)</td>
<td>Age at consultation: 26 years. Minor impairment of motor function. FVC = 80%.</td>
<td>ECG showed LV hypertrophy. QTc: 430ms. QT/PQ = 10.00. LVEDd = 51mm. SF = 27%.</td>
</tr>
<tr>
<td>69</td>
<td>47</td>
<td>BMD, XLDCM/25</td>
<td>[18] (Patient 3); [19] (Patient 1)</td>
<td>Onset of skeletal muscle symptoms: 9 years. Positive family history.</td>
<td>DCM diagnosis. ECG showed infarct pattern of LV posterolateral wall. Family: Affected uncle died of</td>
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<tr>
<td>137</td>
<td>47</td>
<td>BMD, XLDCM/23</td>
<td>[18] (Patient 4)</td>
<td>Onset of skeletal muscle symptoms: 2 years. Awkward gait, could not run as fast as his peers. Diagnosed with limb-girdle muscular dystrophy (incorrectly) and DCM at age 23 years. Age at consultation: 34 years. Clinical status: marked proximal muscular atrophy and weakness, decreased deep tendon reflexes, waddling gait.</td>
<td>Cardiac data at 23 years of age: DCM diagnosis. Cardiac data at 30 years of age: Occasional nocturnal orthopnea, exertional dyspnea, and hemoptysis due to left-sided heart failure.</td>
</tr>
<tr>
<td>152</td>
<td>48</td>
<td>BMD/16</td>
<td>[6]</td>
<td>Age at ECG showed</td>
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<td>194</td>
<td>48</td>
<td>XLDCM/24</td>
<td>[7]</td>
<td>CK = 63U/L. Negative family history.</td>
<td>Onset of congestive heart failure symptoms and DCM diagnosis. EF = 14%. <strong>Cardiac Biopsy:</strong> reduced dystrophin immunoreactivity with an antibody to the rod domain, and irregular staining with antibodies to the amino- and carboxyl-terminal domains.</td>
</tr>
<tr>
<td>190</td>
<td>48-49</td>
<td>BMD/48</td>
<td>[10]</td>
<td>Age at consultation: 48 years. Cramps, myalgia, myoglobinuria, calf hypertrophy. CK = 4700 U/L. Western blot: reduced dystrophin amount (50%) and reduced size (390 kDa).</td>
<td>ECG showed LBBB. Holter ECG showed Lown grade 4b. LVEDV = 229 mL/m². EF = 34%. LV wall motion: diffuse hypokinesia.</td>
</tr>
<tr>
<td>71</td>
<td>48-49</td>
<td>XLDCM/24</td>
<td>[21]</td>
<td>Age at consultation: 24 years. Dyspnea with mild physical activity, no cramps or myalgia associated with exercise. Neurological exam: no weakness or DCM diagnosis. ECG showed Q waves in the inferior and posterior leads, incomplete RBBB. Holter ECG showed sustained ventricular arrhythmias monitoring. LVEDd = 73 mm, EF =</td>
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</table>
muscle wasting or hypertrophy. CK = 540 - 867 U/L. Biopsy showed variable intensity of immunoreactivity among fibers, overall fainter than control. Positive family history.

27%. Angiography showed no coronary artery disease. 

**Cardiac biopsy:** no active myocarditis.

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<tr>
<td>226</td>
<td>48-49</td>
<td>BMD, XLDCM/29</td>
<td>[22] (Patient 2)</td>
<td>Age at consultation: 29 years. Elevated CK. Biopsy showed mild variability of fiber size and moderate interstitial fibrosis; dystrophin staining normal with carboxyl-terminal antibodies. Positive family history.</td>
<td>Cardiac data at 29 years of age: Cardiac symptoms of dyspnea. NYHA II. Marked LV dilation; LVEDV = 176 mL/m², EF = 26%. The patient was treated with high doses of captopril. Cardiac data at 30 years of age: Severe LV dilation; LVEDV = 166 mL/m², EF = 28%. <strong>Cardiac Biopsy:</strong> Positive but faint dystrophin immunofluorescence in all cardiomyocytes. Diffuse but faint over-expression of utrophin.</td>
</tr>
<tr>
<td>153</td>
<td>48-49</td>
<td>BMD, XLDCM/26</td>
<td>[23]</td>
<td>Onset of skeletal muscle symptoms: 32 years. Biopsy showed increased variability of muscle fiber</td>
<td>Cardiac data at 26 years of age: DCM diagnosis. Cardiac data at 27 years of age:</td>
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diameter, rounded and abnormally shaped fibers, internalized myonuclei, slightly proliferated endomysial connective and fat tissue. Dystrophin staining patchy and irregular; reduced size by Western blot (380 kDa). Sarcoglycans and dystroglycan normally expressed. Utrophin detected in some muscle fibers.

Cardiac transplant for end stage DCM.

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<tr>
<td>209</td>
<td>45-55</td>
<td>XLDCM/36</td>
<td>[24]</td>
<td>Age at consultation: 36 years. Normal neurological exam. CK = 1,347U/L.</td>
<td>DCM diagnosis. LV dilatation (LVEDd = 72mm), diffuse hypokinesis of the left ventricular wall motion, reduced cardiac output, EF = 16%. Chest roentgenogram showed slight cardiomegaly, bilateral pleural effusion, and pulmonary congestion. ECG showed sinus tachycardia, poor R-wave</td>
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progression in leads V1-V3 and flat T wave in leads I, aVL, V5 and V6.

*Cardiac biopsy:* myocyte degeneration, irregularly shaped nuclei and interstitial fibrosis.

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<tr>
<td>162</td>
<td>45-55</td>
<td>BMD/69</td>
<td>[16] (Patient 2)</td>
<td>Onset of skeletal muscle symptoms: 59 years. Mild but progressive weakness affecting both arms and legs, difficulty climbing</td>
<td>No cardiac symptoms. Negative cardiac failure, cardiomegaly on chest X-ray. ECG showed prominent</td>
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</table>
hills and stairs, difficulty lifting luggage and standing from a sitting or crouching position. Age at consultation: 69 years. Neurological exam: moderate proximal muscular atrophy and weakness, especially in the lower limbs, positive Gower's sign, waddling gait, no calf hypertrophy. CK = 669 U/L. Biopsy showed fiber size variation, opaque fibers and proliferated connective tissues. Dystrophin staining: faint and discontinuous patchy pattern. Negative family history.

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<tr>
<td>MJ06</td>
<td>45-55</td>
<td>BMD/63</td>
<td>MDC</td>
<td>Diagnosis: 58 years. Positive family history.</td>
<td>Cardiac data at 63 years of age: EF = 15-20%, severe mitral regurgitation. Cardiac data at 64 years of age: ECG showed ventricular tachycardia, widened QRS complex with</td>
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duration about 128msec and frequent PVCs. LV and LA enlargement.

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<tr>
<td>203</td>
<td>48-51</td>
<td>XLDCM/60</td>
<td>[26]</td>
<td>Age at consultation: 65 years. Investigated following incidental finding of elevated CK in his 5-year-old grandson. Normal muscle strength, no trophic changes. Normal CK. Normal EMG. Biopsy was normal apart from occasional small fibers and some internal nuclei. Dystrophin immunostaining: normal in distribution, slightly reduced in amount. Western blot: reduced size of dystrophin. Alpha-sarcoglycan, beta-sarcoglycan, gamma-sarcoglycan, spectrin and merosin showed normal distribution.</td>
<td>Cardiac data at 60 years of age: DCM diagnosis. Severe LV dilation with reduced EF and mitral and aortic regurgitation. Coronary arteriography showed very mild atherosclerosis without significant obstructive lesions. Died suddenly of cardiac arrest at age 68. Family: “Cardiomyopathy” caused the death of his mother and two brothers in their sixth decade.</td>
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<tr>
<td>196</td>
<td>48-51</td>
<td>XLDCM/30</td>
<td>[7] (Patient 4)</td>
<td>CK = 442-515U/L. Negative family history.</td>
<td>Onset of cardiac symptoms. Congestive heart failure diagnosed. EF = 30%. Cardiac Biopsy:</td>
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reduced dystrophin immunoreactivity with an antibody to the rod domain, and irregular staining with antibodies to the amino- and carboxyl-terminal domains.

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<tr>
<td>211</td>
<td>48-52</td>
<td>XLDCM/43</td>
<td>[14] (Patient 3)</td>
<td>Age at consultation: 43 years. No skeletal muscle symptoms. CK = 96U/L. Positive family history.</td>
<td>DCM diagnosis pre-dating cardiac data below. Cardiac data at 43 years of age: ECG showed LBBB. Interventricular septal thickness = 9mm; Posterior wall thickness = 9mm; LVEDd = 72mm; LVEDs = 69mm; SF = 4%.</td>
</tr>
<tr>
<td>200</td>
<td>48-53</td>
<td>XLDCM/50</td>
<td>[7] (Patient 8)</td>
<td>CK below 200U/L. Negative family history.</td>
<td>Onset of cardiac symptoms. Congestive heart failure diagnosed. EF = 30%. <em>Cardiac Biopsy:</em> reduced dystrophin immunoreactivity with an antibody to the rod domain, and irregular staining with antibodies to the amino- and carboxyl-terminal domains.</td>
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<tr>
<td>208</td>
<td>49-51</td>
<td>XLDCM/50</td>
<td>[21] (Patient 2)</td>
<td>Age at consultation: 52 years. No muscle atrophy or pseudohypertrophy, normal muscle strength. CK= 84 U/L.</td>
<td>Cardiac data at 50 years of age: Onset of congestive heart failure symptoms. Cardiac data at 52 years of age: Dyspnoeic at rest, systolic murmur present over the mitral valve. ECG showed negative T waves in leads V5-V6. Dilated LV (LVEDd = 70mm), EF = 20%, dilated RV and both atria. Moderate mitral and tricuspidal valve regurgitation. Cardiac biopsy: significant fibrosis, separating individual cardiomyocytes in some areas, and gross variability of fiber size mainly due to hypertrophic cardiomyocytes; strong and continuous dystrophin staining with antibodies to amino-terminus, mid rod domain, and carboxyl-terminus. Slight</td>
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<tr>
<td>MJ01</td>
<td>51-52</td>
<td>BMD/33</td>
<td>MDC</td>
<td>Onset of skeletal muscle symptoms: birth. Diagnosis: 5 years. Clinical findings: scoliosis since the teenage years, limited mobility at 29 years. Wheelchair bound at 40 years of age.</td>
<td>Cardiac data at 33 years of age: Congestive heart failure, Pacemaker implanted. Cardiac data at 35 years of age: Severe LV dilation with global LV dysfunction, EF = 34%.</td>
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* Age refers to the youngest age in years at which the patient fulfills our criteria for classification as affected with cardiomyopathy.

Abbreviations:

AV: atrioventricular; BMD: Becker muscular dystrophy; CK: creatine kinase; Dx: diagnosis; ECG: electrocardiogram; EDV: end diastole volume; EF: ejection fraction (left ventricle unless otherwise specified); EPSS: end-point septal separation; FVC: forced vital capacity; LA: left atrium; LBBB: left branch bundle block; LV: left ventricle; LVEDd: left ventricular end diastolic diameter; LVEDs: left ventricular end systolic diameter; MDC: muscular dystrophy clinics of Nationwide Children’s Hospital and The Ohio State University; NSI: no specific information; PVC: premature ventricular contraction; RA: right atrium; RBBB: right branch bundle block; RV: right ventricle; SF: shortening fraction; UDP: United Dystrophinopathy Project; WHO: World Health Organization.

Normal values for measured parameters:

CK: 200 U/L (unless otherwise stated); EF: above 55%; EPSS: below 5 mm; FVC: 80%-120% predicted; Intraventricular Septal Thickness: below 12mm; LVEDd: below 58 mm, 2 z scores, or 2 SD; Posterior Wall Thickness: below 12mm; QTc: below 440ms; SF: above 32%; Vignos Scale: described in JAMA (1963), 184:89-96.

Source List:


APPENDIX B

CLINICAL DESCRIPTION OF PATIENTS IN THIS STUDY UNAFFECTED WITH DCM
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<th>Source</th>
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<tbody>
<tr>
<td>216</td>
<td>3-4</td>
<td>BMD/24</td>
<td>[1]</td>
<td>Age at consultation: 24 years. Clinical Severity: severe. Myoglobinuria. FVC = 92%. Positive family history.</td>
<td>ECG showed incomplete RBBB. Holter ECG showed isolated monomorphic PVCs (Lown grade 1). Normal LA volume. LV EDV = 50 mL/m². EF = 67%. Normal LV wall motion. Normal RV EDV. Normal RVEF.</td>
</tr>
<tr>
<td>99</td>
<td>3-9</td>
<td>BMD/6</td>
<td>[1]</td>
<td>Age at consultation: 6 years. Clinical Severity: Mild. Skeletal muscle symptoms are present. CK = 1770 U/L. Western blot: reduced dystrophin expression (60%) and size (370 kDa). Negative family history.</td>
<td>Normal ECG. Normal LA volume. LV EDV = 37 mL/m². EF = 60%. Normal LV wall motion. RV EDV=41 mL/m². RVEF = 68%. Normal RV wall motion.</td>
</tr>
<tr>
<td>AH02</td>
<td>5</td>
<td>BMD/14</td>
<td>MDC</td>
<td>Onset of skeletal muscle symptoms: 10 years. Frequent falling, difficulty climbing stairs, calf hypertrophy, positive Gower's sign. CK = 7651 U/L. Negative family history.</td>
<td>LVEDd = 0.5sd, EF = 60%, SF = 40%.</td>
</tr>
</tbody>
</table>

**Group 2 Patients.**

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<tbody>
<tr>
<td>SL188</td>
<td>45</td>
<td>BMD/15</td>
<td>UDP</td>
<td>Onset of skeletal muscle symptoms: 3 years. Diagnosis: 6 years. Weakness, calf hypertrophy and Gower's sign. Began wheelchair use at 12 years of age. Vignos Scale: upper = 1 / lower = 2.</td>
<td>EF = 63%, SF = 35%.</td>
</tr>
<tr>
<td>217</td>
<td>45-47</td>
<td>BMD/28</td>
<td>[1] (Patient 24)</td>
<td>Age at consultation: 28 years. Clinical Severity: Moderate.</td>
<td>ECG showed T wave changes. Normal Holter ECG. Normal LA volume. LV EDV = 69 mL/ m². EF = 55%. Normal LV wall motion. RV EDV = 66 mL/ m². RVEF = 64%.</td>
</tr>
<tr>
<td>106</td>
<td>45-47</td>
<td>BMD/33</td>
<td>[1] (Patient 28)</td>
<td>Age at consultation: 33 years. Clinical Severity: Moderate. Biopsy showed Normal ECG. Holter ECG showed isolated monomorphic PVCs (Lown grade 1). LA volume =</td>
<td></td>
</tr>
</tbody>
</table>
small number of dystrophin negative fibers. Western Blot: reduced amount (50%) and size (380 kDa). Positive family history.

29 mL/ m². LV EDV = 44 mL/ m². EF = 55%. Normal LV wall motion. RV EDV = 52 mL/ m². RVEF = 52%.

<table>
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<th>Dx/Age*</th>
<th>Source</th>
<th>Clinical information</th>
<th>Cardiac information</th>
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</thead>
<tbody>
<tr>
<td>213</td>
<td>45-47</td>
<td>BMD/6</td>
<td>[1]</td>
<td>(Patient 2) Age at consultation: 6 years. Clinical Severity: Mild. CK = 5000 U/L. FVC = 56% (moderate restrictive respiratory insufficiency).</td>
<td>Normal ECG. Normal LA volume. LV EDV = 67 mL/ m². EF = 64%. Normal LV wall motion. RV EDV = 46 mL/ m². RVEF = 57%. Normal RV motion.</td>
</tr>
<tr>
<td>SL382</td>
<td>45-47</td>
<td>BMD/19</td>
<td>UDP</td>
<td>Onset of skeletal muscle symptoms: 1.5 years. Diagnosis: 9 years. Toe walking, myalgia, cramping. Vignos Scale: upper = 1 / lower = 1.</td>
<td>EF=57%, SF=32%.</td>
</tr>
<tr>
<td>MJ09</td>
<td>45-47</td>
<td>BMD/24</td>
<td>MDC</td>
<td>Diagnosis: age 4 years. Biopsy showed muscle fiber size variation and necrosis. Elevated</td>
<td>EF = 60%, SF = 42.5%.</td>
</tr>
<tr>
<td>ID</td>
<td>Deleted Exons</td>
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<td>Clinical information</td>
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</tr>
<tr>
<td>AH03</td>
<td>45-47</td>
<td>BMD/10</td>
<td>MDC</td>
<td>Onset of skeletal muscle symptoms: 6 years. CK = 5650 U/L. Mild calf hypertrophy, mild lordosis, kyphosis, scoliosis, toe walking, positive Gower's sign, weakness, chronic pulmonary disease, developmental motor delay. Positive family history.</td>
<td>EF = 55%, SF = 33%</td>
</tr>
<tr>
<td>AH07</td>
<td>45-47</td>
<td>BMD/9</td>
<td>MDC</td>
<td>Onset of skeletal muscle symptoms: 5 years. Muscle fatigue, calf hypertrophy, and hip pain. CK=18137 U/L. Biopsy showed no inflammation, mild fatty replacement and mild fibrosis. Staining for dystrophin, sarcoglycans, beta-dystroglycan and laminin showed strong undisrupted sarcolemmal staining. Western Blot: several</td>
<td>LVEDd = 0.9 sd, EPSS = 2mm, SF = 40%, EF = 60%.</td>
</tr>
<tr>
<td>ID</td>
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<td>Dx/Age*</td>
<td>Source</td>
<td>Clinical Information</td>
<td>Cardiac Information</td>
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</tr>
<tr>
<td>KJ03</td>
<td>45-47</td>
<td>BMD/45</td>
<td>MDC</td>
<td>Onset of skeletal muscle symptoms: 8 years. Leg weakness, difficulty climbing stairs or arising from the floor. Diagnosis: 37 years. Clinical status: ambulant at age 47 years, wide-based gait with exaggerated lordosis and some waddle. Positive family history.</td>
<td>Echocardiography normal. Stress test negative.</td>
</tr>
<tr>
<td>AH10</td>
<td>45-48</td>
<td>BMD/15</td>
<td>MDC</td>
<td>Diagnosis: 10 months of age. Onset of skeletal muscle symptoms: 8 years. Calf hypertrophy, muscle cramps. CK = 5 210 U/L. Positive family history.</td>
<td>EF = 58%, SF = 32.23%.</td>
</tr>
<tr>
<td>214</td>
<td>45-48</td>
<td>BMD/10</td>
<td>[1] (Patient 4)</td>
<td>Age at consultation: 10 years. Clinical Severity: Mild. Myoglobinuria. CK = 7300 U/L. FVC = 69% (mild restrictive)</td>
<td>ECG showed R/S&gt;1. Normal Holter ECG. Normal LA volume. LV EDV = 50 mL/ m². EF = 62%. Normal LV wall motion. RV</td>
</tr>
<tr>
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<td>Source</td>
<td>Clinical information</td>
<td>Cardiac information</td>
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<tr>
<td>181</td>
<td>45-48</td>
<td>BMD/20</td>
<td>[2]</td>
<td>Age at consultation: 20 years. Cramps, myalgia, myoglobinuria, calf hypertrophy. CK = 1774 U/L. Western blot: normal dystrophin amount but reduced size (360 kDa).</td>
<td>ECG showed left anterior fascicular block. Normal Holter ECG. LV EDV = 72 mL/ m². EF = 63%.</td>
</tr>
<tr>
<td>MJ26</td>
<td>45-48</td>
<td>BMD/29</td>
<td>MDC</td>
<td>Shoulder girdle weakness and difficulty running since early childhood. Neurological exam at 18 years: CK = 2500-3300 U/L. Biopsy: fiber size variation, atrophic</td>
<td>LVEDd = 0.5sd, SF = 37%, EF = 58%, no mitral regurgitation.</td>
</tr>
</tbody>
</table>
fibers, mild to moderate increase of internal nuclei. Western Blot: reduced dystrophin amount.

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<th>ID</th>
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<th>Source</th>
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<th>Cardiac information</th>
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<tbody>
<tr>
<td>103</td>
<td>48</td>
<td>BMD/13</td>
<td>[1] (Patient 10)</td>
<td>Age at consultation: 13 years. Clinical Severity: Mild. Skeletal muscle symptoms are present. CK = 3910 U/L. Western blot: reduced dystrophin expression (80%) and size (390 kDa). Positive family history.</td>
<td>ECG showed T wave changes and R/S&gt;1. Normal Holter ECG. Normal LA volume. LV EDV = 49 mL/ m². EF = 58%. RV EDV = 55 mL/ m². RVEF = 56%.</td>
</tr>
<tr>
<td>184</td>
<td>48</td>
<td>BMD/24</td>
<td>[2] (Patient A17)</td>
<td>Age at consultation: 24 years. Cramps, myalgia, myoglobinuria, calf hypertrophy. CK = 18 U/L. Western blot:</td>
<td>ECG showed R/S&gt;1. Normal Holter ECG. LV EDV = 88 mL/ m². EF = 56%.</td>
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reduced dystrophin amount (60%) and reduced size (395 kDa).

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<th>Source</th>
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<th>Cardiac information</th>
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<tbody>
<tr>
<td>108</td>
<td>48-49</td>
<td>BMD/14</td>
<td>[1] (Patient 12)</td>
<td>Age at consultation: 14 years. Clinical Severity: Mild. Skeletal muscle symptoms are present. FVC = 105%. Biopsy showed many dystrophin negative fibers. Western blot: reduced dystrophin</td>
<td>ECG showed incomplete RBBB. Holter ECG showed Lown grade 1. Normal LA volume. LV EDV = 77 mL/ m². EF = 65%. Normal LV wall motion. RV EDV = 110 mL/ m². RVEF = 56%. RV wall motion showed septal akinesia.</td>
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expression (50%) and size (390 kDa). Positive family history.

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<tr>
<th>ID</th>
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<th>Source</th>
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<th>Cardiac information</th>
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<tr>
<td>180</td>
<td>48-49</td>
<td>BMD/18</td>
<td>[2] (Patient A11)</td>
<td>Age at consultation: 18 years. CK = 6427 U/L. Cramps, myalgia, myoglobinuria, calf hypertrophy. Western blot: reduced amount (50%) and size (390 kDa). ECG showed incomplete RBBB. Holter ECG showed isolated monomorphic PVCs (Lown grade 1). LV EDV = 86 mL/ m². EF = 58%. Normal LV wall motion.</td>
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<tr>
<td>100</td>
<td>45-51</td>
<td>BMD/10</td>
<td>[1] (Patient 5)</td>
<td>Age at consultation: 10 years. Clinical Severity: Mild. Skeletal muscle symptoms are present. CK = 1616 U/L. Western blot: normal dystrophin expression levels but reduced size (360 kDa). Negative family history. ECG showed R/S&gt;1. Holter ECG showed isolated monomorphic PVCs (Lown grade 1). Normal LA volume. LV EDV = 63 mL/ m². EF = 69%. Normal LV wall motion. RV EDV = 60 mL/ m². RVEF = 68%. Normal RV wall motion.</td>
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<tr>
<td>SL54</td>
<td>45-51</td>
<td>BMD/13</td>
<td>UDP</td>
<td>Age of skeletal muscle symptom onset: 4 years. Diagnosis: 11 years. Myalgia, cramping. Vignos Scale: upper = 1 / lower = 1. EF = 65%, SF = 34%.</td>
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<tr>
<td>ID</td>
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<td>Dx/Age*</td>
<td>Source</td>
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<tr>
<td>185</td>
<td>45-52</td>
<td>BMD/29</td>
<td>[2]</td>
<td>(Patient A18)</td>
<td>Age at consultation: 29 years. Cramps, myalgia, myoglobinuria, calf hypertrophy. CK = 2100 U/L. Western blot: normal dystrophin amount but reduced size (360 kDa). Normal ECG. LVEDV = 53 mL/m². EF = 61%.</td>
</tr>
<tr>
<td>178</td>
<td>45-55</td>
<td>BMD/12</td>
<td>[2]</td>
<td>(Patient A5)</td>
<td>Age at consultation: 12 years. Clinical Severity: Mild. Cramps, myalgia, myoglobinuria, calf hypertrophy. CK = 6000 U/L. FVC normal. Western blot: reduced dystrophin amount (40%) and reduced size (340 kDa). Negative family history. ECG showed R/S&gt;1 and incomplete LBBB. Holter ECG showed isolated monomorphic PVCs (Lown grade 1). Normal LA volume. LV EDV = 50 mL/m². EF = 61%. Normal LV wall motion. RV EDV = 63 mL/m². RVEF = 60%. Normal RV wall motion.</td>
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<td>Source</td>
<td>Clinical information</td>
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<td>Source</td>
<td>Clinical Information</td>
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* Age refers to the age in years of the patient at the last reported cardiac evaluation with cardiac findings that fulfill our criteria for a classification of non-affected with cardiomyopathy.

Abbreviations:
- **BMD**: Becker muscular dystrophy; **CK**: creatine kinase; **Dx**: diagnosis; **ECG**: electrocardiogram; **EDV**: end diastole volume; **EF**: ejection fraction (left ventricle unless otherwise specified); **EMG**: electromyography; **EPSS**: end-point septal separation; **FVC**: forced vital capacity; **LA**: left atrium; **LBBB**: left branch bundle block; **LV**: left ventricle; **LVEDd**: left ventricular end diastolic diameter; **MDC**: muscular dystrophy clinics of Nationwide Children’s Hospital and The Ohio State University; **PVC**: premature ventricular contraction; **RBBB**: right branch bundle block; **RV**: right ventricle; **SF**: shortening fraction; **UDP**: United Dystrophinopathy Project.

Normal values for measured parameters:
- **CK**: 200 U/L (unless otherwise stated); **EF**: above 55%; **EPSS**: below 5 mm; **FVC**: 80%-120% predicted; **LVEDd**: below 58 mm, 2 z scores, or 2 SD; **SF**: above 32%; **Vignos Scale**: described in JAMA (1963), 184:89-96.

Source List:


- Duchenne muscular dystrophy
- Becker muscular dystrophy
- X-linked DCM\(^1,2\)
- Retinal neurotransmission defect\(^2\)
- Mental retardation\(^2\)
- Psychiatric disturbances\(^2\)

\(^1\) Can manifest without any musculoskeletal manifestation.
\(^2\) Usually present with DMD and/or BMD.

Table 1: Clinical Phenotypes Resulting From Mutations in the Dystrophin Gene
**Genotype**
Theoretical Definition: The versions of a gene for a given trait carried by an organism (Klug, Cummings & Spencer, 2006).

In-frame mutations of the dystrophin gene result in dysfunctional or partial production of dystrophin protein. These mutations are disruptions in the DNA sequence (i.e., base pair substitution) that allow the codon reading frame to be maintained. Therefore, even though an incorrect amino acid is encoded at that mutated region, the downstream codons remain unaffected. The consequence to the protein is not as detrimental as that of frame-shift mutation.

Frame-shift mutations in the dystrophin gene result in no production of dystrophin protein in the cell. These mutations are disruptions in the DNA sequence that affect all downstream codons, in that incorrect or no amino acids can be encoded beginning from the mutation point. The end result is that a protein cannot be produced.

Exons represent the genomic DNA sequences that are retained and expressed in the final mRNA transcript (Klug, Cummings & Spencer, 2006). There are 79 exons in the dystrophin gene. Exon deletions are DNA deletion mutations that result in the removal of a section of the coding sequence.

**Protein Structure**
Theoretical Definition: A protein is a molecule composed of multiple amino acids. The accurate conformation of its 3-dimensions relies heavily on the composing amino acids and is essential for normal function. The precursors of a protein are polypeptides, which are assembled during the transcription process based on the RNA sequence. Because RNA is synthesized from a DNA template, the correct original DNA sequence is crucial in the making of a protein (Klug, Cummings & Spencer, 2006).

Dystrophin Protein Defect refers to the state of dystrophin protein dysfunction, such as reduced molecular weight or lower quantity expressed in the myofiber.

Dystrophin Protein Deficiency refers to the state of dystrophin protein absence.

Spectrin-like repeats are the structural units that make up the central rod domain of the dystrophin protein. There are 24 of spectrin-like repeats, and their structure and composition rely on the encoding exons. For example, exons 48 and 49 encode for the 19th spectrin-like repeat, and when exon 48 deletion...
mutation occurs, only partial portion of the 19th spectrin-like repeat can be made. The structure of spectrin-like repeats can be classified as in-phase and out-of-phase, as described earlier.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Theoretical Definition:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Theoretical Definition:</strong></td>
<td>The observable features of an organism reflecting its genetic makeup, or the genotype (Klug, Cummings &amp; Spencer, 2006).</td>
</tr>
<tr>
<td><strong>Phenotype</strong></td>
<td>The primary clinical characteristics reflecting dystrophin defect versus deficiency is the patient’s musculoskeletal symptoms. <strong>Milder musculoskeletal symptoms</strong> are found in BMD patients, presumably due to their abnormal yet present production of dystrophin protein. When the BMD patient’s symptoms of dystrophinopathy are predominantly cardiac pathology, the patient is classified as having X-linked DCM in this study. <strong>Severe musculoskeletal symptoms</strong> are seen in DMD patients, 99% of whom do not express dystrophin at all in the muscle (Hoffman, 1993). <strong>Age of DCM manifestation</strong> is the outcome phenotype for dystrophin genetic mutation. Age of DCM manifestation is not necessarily the true onset of the disease; rather, it reflects closely to the time when cardiac symptoms are noted.</td>
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Table 2: Constructs and concepts in the theoretical model.
<table>
<thead>
<tr>
<th></th>
<th>Non-Affected</th>
<th>Affected</th>
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<tbody>
<tr>
<td>Shortening Fraction</td>
<td>≥33%</td>
<td>&lt;32%</td>
</tr>
<tr>
<td>Ejection Fraction</td>
<td>≥55%</td>
<td>&lt;55%</td>
</tr>
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<td>Left Vent. Internal Diastolic Dimension</td>
<td>≤58mm or 2 SD/z scores</td>
<td>&gt;58mm or 2 SD/z scores</td>
</tr>
<tr>
<td>E-Point Septal Separation</td>
<td>≤5mm</td>
<td>&gt;5mm</td>
</tr>
<tr>
<td>Cardiac History</td>
<td>None</td>
<td>Heart block, dilated cardiomyopathy, heart failure, heart transplant</td>
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</tbody>
</table>

Table 3. Cardiac function categories.
Table 4: Patient numbers according to diagnosis and source, with median ages based on cardiomyopathy categorization. For BMD patients from both sources, the median ages for the cardiomyopathic and the non-cardiomyopathic were significantly different (p<0.001). For all cardiomyopathic patients, regardless of clinical diagnoses and sources, there was no statistically significant difference in median age. Similarly, for all non-cardiomyopathic patients, there was no statistically significant difference in median age.
<table>
<thead>
<tr>
<th>Exon Deletion</th>
<th>Phasing of spectrin-like repeats</th>
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<tbody>
<tr>
<td>45</td>
<td>Out-of-phase</td>
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<tr>
<td>45-46</td>
<td>In-phase</td>
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<tr>
<td>45-47</td>
<td>Out-of-phase</td>
</tr>
<tr>
<td>45-48</td>
<td>In-phase</td>
</tr>
<tr>
<td>45-49</td>
<td>Out-of-phase</td>
</tr>
<tr>
<td>46-49</td>
<td>In-phase</td>
</tr>
<tr>
<td>47</td>
<td>Out-of-phase</td>
</tr>
<tr>
<td>47-48</td>
<td>In-phase</td>
</tr>
<tr>
<td>48</td>
<td>Out-of-phase</td>
</tr>
<tr>
<td>48-49</td>
<td>In-phase</td>
</tr>
</tbody>
</table>

Table 5: Phasing consequences of different exon deletion mutations.
<table>
<thead>
<tr>
<th>Group</th>
<th>Median Age (years)</th>
<th>Group</th>
<th>Median Age (years)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2 (in-phase)</td>
<td>36.5</td>
<td>Vs. Group 1</td>
<td>22.5</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vs. Group 3</td>
<td>46.5</td>
<td>0.057</td>
</tr>
<tr>
<td>Group 2 (out-of-phase)</td>
<td>25.5</td>
<td>Vs. Group 1</td>
<td>22.5</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vs. Group 3</td>
<td>46.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 6: Pair-wise comparison of cardiomyopathic patient groups based on the location of the deletion mutation and the effects on spectrin-like repeat phasing. Significant p values are bolded and italicized. The Mann-Whitney U test was used for statistical comparisons. The median age of cardiac manifestations is indicated for each group.
Figure 1: Gower’s maneuver. DMD patients face the floor, plant their feet widely apart, raise their buttocks first, and use their hands to “walk” up the legs. Modified from Muscular Dystrophy Association (2006).
Figure 2: Anatomy of DCM. Left: normal heart; Right: DCM.
Figure 3: The architecture of dystrophin together with other proteins on the sarcolemma. Graph modified from Rodino-Klapac, Chicoine, Kaspar & Mendell (2007).
Figure 4: Graphic representation of the dystrophin gene and protein. There are three binding domains as labeled (top). The first panel indicates the protein structure. The second panel indicates the 79 exons of the dystrophin gene. The colors of the exons coordinate with regions of the protein the exons are predicted to encode. The third panel illustrates the flexibility of the dystrophin protein. **N**: N-terminus; **R**: spectrin-like repeats; **H**: hinges; **CR**: cysteine-rich domain; **CT**: C-terminus.
Figure 5: Known Pathways through which abnormal dystrophin leads to cardiomyocyte death. DAPC: Dystrophin-associated Protein Complex; CK: creatine kinase; Ca$^{++}$: calcium.
Figure 6: Progression from cardiomyocyte death to DCM.

CARDIOMYOCYTE DEATH

\[ \downarrow \] Inflammatory Response
\[ \downarrow \] Fibrosis Formation
\[ \downarrow \] Stretch and Thinning of Fibrotic Region
\[ \downarrow \] Dilation of the Heart

\[ \uparrow \] Left Ventricular Volume
\[ \uparrow \] Wall Stress
\[ \downarrow \] Diastolic Relaxation
\[ \downarrow \] Contractility
\[ \downarrow \] Cardiac Output
Mitral Valve Regurgitation
Figure 7: Modeling of the rod region of spectrin. Each repeat contains helices number 2, number 3 and the number 1 of the next unit. When folded into a three-dimensional structure, the linker sequence forms a flexible loop. Figure modified from Parry, Dixon and Cohen (1992).
Dystrophin Gene Mutations

GENOTYPE

PROTEIN STRUCTURE

PHENOTYPE

In-frame mutation

Frame-shift mutation

Exon Deletion

Dystrophin Protein Defect

Dystrophin Protein Deficiency

Spectrin-like Repeats Structure

Milder Musculoskeletal Symptoms

Severe Musculoskeletal Symptoms

Age of DCM Manifestation

BMD

XLDCM

DMD

In-phase

Out-of-phase

Subject Selection

Data Analysis

Main Stance

(Monaco et al., 1988)

(Harper et al., 2002)

Figure 8: Theoretical model guiding this dissertation study.
Figure 9: Graphic representation of in-phase vs. out-of-phase dystrophin constructs. △17-48 indicates deletions of exons 17-48, and △H2-R19 indicates deletions from Hinge 2 to spectrin-like repeat 19. Top panel is the wild type dystrophin. Numbers on the right indicate molecular weights of the coinciding dystrophin. Figure modified from Harper et al. (2002). SR: Spectrin-like repeat.
Figure 10: Patient grouping by dystrophin protein domain.
Figure 11: Schematic representations of the normal structure of the spectrin-like repeats in dystrophin.

A. A long α-helical segment (Helix 1) alternates with a short Helix 2 of approximately half the length of Helix 1. Variable-length linkers provide a direction-reversing turn at the end of each helix. Because the Helix 2 structures are anti-parallel to the Helix 1 structures and therefore backtrack along the rod axis, every Helix 1 overlaps its N and C terminal Helix 1 neighbors by approximately half its length.

B. Representation of a five-repeat segment of the rod domain of dystrophin based on the Cross et al. (1990) model. The structure is oriented with the N-terminal to the lower left, and the C-terminal to the upper right. The repeating motif is Helix 1 (orange-red) followed by Helix 2 (gray), with each Helix 1 protruding into the adjacent repeat motif creating an interlocking, nested structure.
Figure 12: Mutation distribution for the cardiomyopathic, grouped by diagnoses and sources. Each horizontal bar represents an exon deletion mutation. The number in parenthesis is the number of patient with that specific deletion.
Figure 13: Distribution of mutations for cardiomyopathic patients by group.
Figure 14: Age distribution of each mutation group based on cardiac involvement.
Re-arrangement:
An in-phase spectrin-like repeat

Re-arrangement:
An out-of-phase spectrin-like repeat

Figure 15: Distribution of mutation by group.
Figure 16: Median ages for Group 2 mutation based on spectrin-like repeat phasing.
Figure 17: Predicted models of dystrophin spectrin-like repeat structure based on in-phase (A) and out-of-phase (B & C) deletions. C: A new hinge is created as a result of exon 48 deletion.
Figure 18. Distribution of dystrophin point mutations with XLDPM clinical diagnosis.
Figure 19. Human dystrophin 18Lysine>asparagine mutation. This mutation affects the third position (black highlighting) of a Calponin homology (CH) domain (white background). Lysine 18 is perfectly conserved in Dystrophin from fish to humans and in all known species with Utrrophin (chick and mammals). Sequence flanking the CH domain is shown with gray background. Yellow highlighting shows amino acid divergence from the human sequence.
Figure 20: Human dystrophin 279threonine>alanine mutation. This mutation is in an evolutionarily conserved residue of the dystrophin Hinge 1 region domain. The multiple sequence alignment of dystrophin from fish to human shows positions divergent from human in yellow. We found perfect conservation of threonine 279 (brown highlighted position) in dystrophin from fish to humans. Threonine 279 is within one of two very highly conserved short sequences, both apparent beta strands (secondary structure elements are predicted below the sequence alignment using Pele and Phyre programs). The full Pfam domain is 51% conserved in zebrafish; there is no highly similar sequence in any other protein. The Pfam domain is entirely within a supposed hinge. However, it does not appear to be a single flexible loop. Two apparent loops are serine-and proline-rich, and highly charged (mapped on the human sequence, top).
Figure 21: Human dystrophin 1672 asparagine>lysine mutation. Asparagine 1672 (highlighted black) is conserved in all vertebrate dystrophins including the fish Tetraodon and Fugu, but not zebrafish, which has lysine. Asparagine 1672 is also widely conserved in the closely related protein Utrophin, but not in fish, which have serine at that position. The entire domain (white, with flanking sequence in gray) is very highly conserved throughout evolution. Sequence that diverges from human is highlighted yellow.
Figure 22: Human dystrophin 3228 phenylalanine>leucine mutation. The second EF hand domain (shown as white sequence, with flanking sequence in gray) is a dystroglycan-binding domain. It is strikingly highly conserved from fish to human (sequence divergence from human is shown in yellow). Phenylalanine 3228 is perfectly conserved in dystrophin from diverse species, including fish - except hagfish (not shown).
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