INVITED REVIEW

CARDIOMYOPATHY OF DUCHENNE MUSCULAR DYSTROPHY: CURRENT UNDERSTANDING AND FUTURE DIRECTIONS

CHRISTOPHER F. SPURNEY, MD

Division of Cardiology, Research Center for Genetic Medicine, Children's National Medical Center, 111 Michigan Avenue NW, Washington, DC 20010, USA

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ABSTRACT: Duchenne muscular dystrophy (DMD) is the most common and severe form of muscular dystrophy and occurs in 1 in 3500 male births. Improved survival due to improvements in clinical care of the musculoskeletal and respiratory systems has led to an increased incidence of cardiomyopathy. Cardiac-related deaths are now seen in approximately 20% of DMD patients. Our current understanding of DMD cardiomyopathy has increased significantly over the past 10 years, but further research is required to improve cardiac treatment and outcomes in DMD. This review provides a summary of the current literature and discussion of potential new therapies for DMD cardiomyopathy.

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Duchenne muscular dystrophy (DMD) is the most common and severe form of muscular dystrophy and occurs in 1 in 3500 male births. Due to a mutation in the protein dystrophin, patients with DMD develop progressive muscle weakness and lose the ability to walk between 10 and 12 years of age. In the second decade of life, respiratory and cardiac muscle diseases become significant contributors to disease progression and quality of life. Improvements in the treatment of respiratory muscle disease, including assist devices and mechanical ventilation, allow patients to live longer with improved respiratory function. Eagle et al. showed that patients who undergo spinal surgery and nocturnal ventilation have a mean survival of 30 years, compared with patients who are only ventilated (22.2 years).[1,2] However, this increased lifespan has allowed cardiac disease to emerge as a major cause of patient morbidity and mortality. Cardiomyopathy is now a leading cause of death in DMD patients.[1] As this paradigm switch continues, new focus on the diagnosis and treatment of cardiac disease in DMD is essential. This review focuses on our current understanding of the diagnosis and treatment, along with potential new therapies, for the cardiomyopathy of DMD.

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ATII, angiotensin II; BMD, Becker muscular dystrophy; BNP, brain natriuretic protein; CMR, cardiac magnetic resonance; DMD, Duchenne muscular dystrophy; DTI, Doppler tissue imaging; ECG, electrocardiogram; EF, ejection fraction; GRMD, Golden Retriever muscular dystrophy; iPS, induced pluripotent stem cells; LGE, late gadolinium enhancement; MPI, myocardial performance index; PCR, polymerase chain reaction; PMI, point of maximal impulse; PMO, phosphorodiamidate morpholino oligomer; rAAV, recombinant adeno-associated virus; SF, shortening fraction; TGF-β, transforming growth factor-beta

Key words: • • •

Correspondence to: C. F. Spurney; e-mail: cspurney@cnmc.org

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PATHOGENESIS

An in-depth discussion of the pathophysiology of DMD is beyond the scope of this review. In skeletal muscle, the absence of dystrophin leads to loss of membrane integrity and increased susceptibility to damage from muscle contractions. This damage leads to an influx of extracellular calcium, which can activate proteases within the cell. Protease activity ends in myocyte cell death, necrosis, inflammation, and replacement fibrosis. In the heart, along with membrane integrity, the loss of dystrophin affects L-type calcium channels and mechanical stretch–activated receptors.[3,4] These abnormalities contribute to increased intracellular calcium. The excessive calcium can stimulate further intracellular calcium release and activation of calpains, proteases that degrade the contractile proteins.[5] As with skeletal muscle, this leads to the same pathological cycle of infiltrating inflammatory cells and fibroblasts causing myocardial cell death and fibrosis. The loss of viable myocardium leads to increased wall stress, increased myocardial oxygen demand within viable myocardium, continued cardiomyocyte death, and further fibrosis. Based on a 2006 scientific statement by the American Heart Association, cardiomyopathy is defined as a "heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical dysfunction."[6] Hence, once a DMD patient develops a significant amount of fibrosis leading to decreased function, clinically the patient has cardiomyopathy.

TIMING OF DMD CARDIOMYOPATHY

Skeletal Muscle Strength and Exercise. Studies have shown that the timing and severity of cardiomyopathy is unrelated to the severity of skeletal muscle involvement in DMD. However, could the opposite be true? Could the onset of cardiomyopathy be earlier in patients who maintain skeletal muscle strength? Becker muscular dystrophy (BMD) is due to the partial loss of dystrophin protein, and these patients have milder skeletal muscle symptoms. In BMD, cardiomyopathy can be the presenting symptom, and it is exacerbated by the slower decline of skeletal muscle strength. Heart

failure can be severe enough to require transplantation.[7,8] A similar example is X-linked cardiomyopathy. These patients have loss of dystrophin in the cardiac muscle only and present with severe dilated cardiomyopathy and normal skeletal muscle strength at a young age.[9,10] The current hypothesis is that improved muscle strength increases the workload on the heart and leads to earlier development of cardiomyopathy.[8] Recent exercise studies in the dystrophin-deficient *mdx* mouse showed increased cardiac fibrosis after voluntary wheel or treadmill exercise in both young and older mice.[11–13] It is known that the benefits of steroid therapy on skeletal muscle in DMD allows patients to ambulate longer.[14] As will be discussed later, there are also studies that show steroids prevent progression of cardiac disease. Currently, there is no evidence to suggest that the onset of cardiomyopathy occurs earlier in the current steroid era secondary to prolonged ambulation. However, further studies are needed to better understand the effects of exercise type and intensity on skeletal and cardiac muscle function in DMD patients.

Deletion Type. The large dystrophin gene (14-kb transcript) is composed of four protein domains: amino-terminus (exons 2–7); rod structure with hinges (exons 8–64); cysteine-rich (exons 65–69); and carboxyl-terminus (exons 70–79).[15] There is significant interest in the relationship between cardiomyopathy and type of dystrophin gene deletion. Nigro et al. showed a close linkage between severe cardiomyopathy and deletions encompassing exons 48–49 in both DMD and BMD patients.[16] A study of BMD patients showed that specific mutations influence the development of cardiomyopathy.[15] Patients with deletions in exons 2–9 had the earliest onset of cardiomyopathy. Patients with deletions in exons 45–49 (out-of-phase mutations in the rod domain) showed earlier cardiomyopathy compared with patients who had deletions in exons 50–51 (in-phase mutations of the rod domain). Jefferies et al. also showed that DMD and BMD patients with deletions in exons 51 and 52 had a decreased risk of cardiac involvement. Also, the earliest onset of cardiomyopathy was seen in deletions involving exons 12 and 14–17.[17] The mechanism of differential effect of these mutations is not yet clear, but it is important to note that exon boundaries need not specifically correlate with physical protein boundaries and can have significant functional implications at the protein level.[15] Continued analysis of deletion type and cardiomyopathy disease course, especially in new clinical trials, will hopefully begin to show strong correlations that could direct therapeutic interventions in the future.

Infectious. Early presentation of severe cardiomyopathy in DMD could be secondary to another diagnosis. Mavrogeni et al. reported viral myocarditis in 4 of 6 DMD patients with fibrosis that was seen on cardiac magnetic resonance (CMR) imaging. Polymerase chain reaction (PCR) confirmed the presence of cytomegalovirus, parvovirus B19, and Coxsackie B viruses in 3 of the patients. These patients developed severe left ventricular dilation and decreased function over the following year, and 2 died within 2 years.[18] Previous work by Xiong et al. in *mdx* mice showed that infection with Coxsackie B3 enterovirus led to greater viral replication and more severe cardiomyopathy compared with normal control mice.[19] Thus, DMD patients potentially show increased susceptibility to viral myocarditis, resulting in severe cardiomyopathy at a young age. This possibility should be considered in the proper clinical presentation, and appropriate diagnostic testing should also be considered.

DIAGNOSIS

Although all DMD patients >18 years of age will show evidence of cardiac muscle disease, only slightly more than half will complain of any symptoms.[20] Due to the inability to ambulate at this age, the common symptom of exercise intolerance is often not appreciated by DMD patients. Instead, patients experience vague symptoms, including sleep disturbances, loss of appetite, nausea, abdominal pain or fullness, increased cough or secretions, and weight loss. Patients can also experience more classic cardiac symptoms, including chest pain, palpitations, dizziness and syncope. These are usually more related to the presence of arrhythmias rather than heart failure. Consequently, the physician must ask specific questions regarding seemingly small changes in sleep or daily activities.

Physical Examination. Cardiac physical findings can provide initial clues to the presence and extent of cardiac disease. Vital signs often include resting tachycardia in DMD patients. On examination of the neck, jugular venous distention can be present. On chest palpation, displacement of the point of maximal impulse (PMI) inferolaterally is due to an enlarged left ventricle. The PMI can also be displaced secondary to scoliosis. On auscultation, there is usually a regular rhythm with a normal S1 and S2. Irregular rhythms are usually associated with atrial tachyarrhythmias or ventricular ectopy. An S3 gallop can be heard during acute congestive heart failure and an S4 gallop can be heard secondary to left ventricular dysfunction. Systolic ejection flow murmurs and systolic regurgitant murmurs, usually due to mitral regurgitation from left ventricular dilation, can be associated

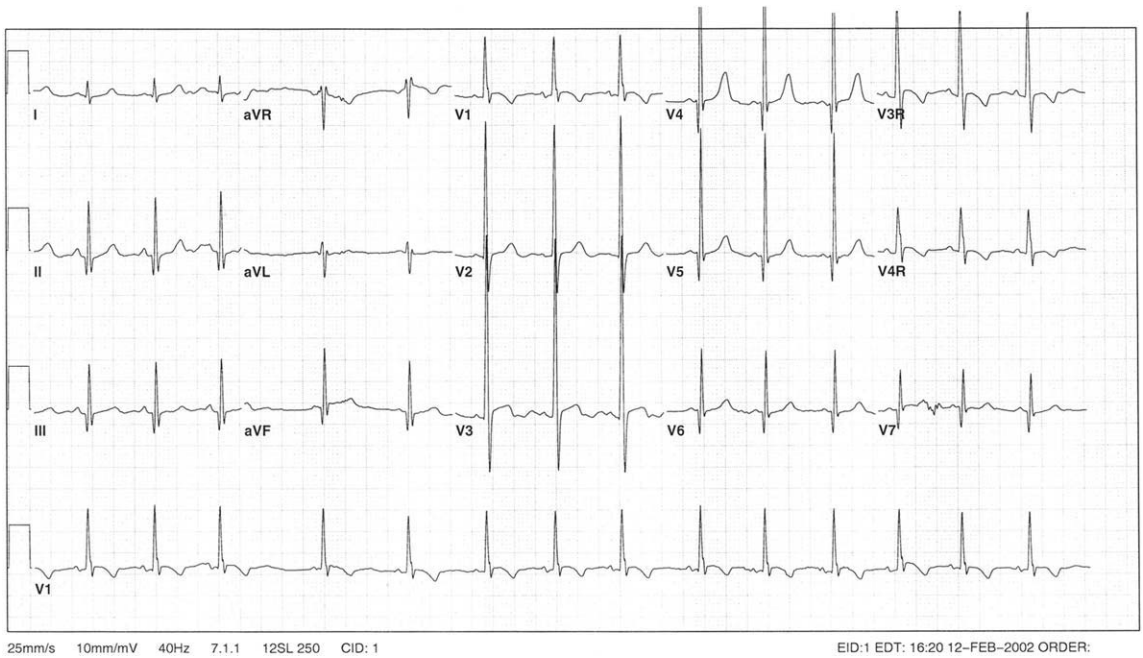

FIGURE 1. Electrocardiogram tracing of an 8-year-old DMD patient. This shows common features of DMD including resting tachycardia with a heart rate of approximately 90 beats per minute, increased R-wave amplitudes in leads V1, V2, and V3, and Q waves in the lateral and inferior leads (II, III, aVF, V4, V5, V6).

with DMD. Pulmonary auscultation will show decreased breaths and rales at the bases bilaterally. Hepatomegaly can be found on abdominal examination, but the liver is usually difficult to palpate due to positioning and scoliosis. Examination of the extremities can show dependent edema when heart failure develops.

Electrocardiography. Sinus tachycardia is found in a majority of DMD patients, beginning during childhood and occurring even when these patients are immobile.[21–24] Abnormally tall R waves in leads V1–V3 are also found in DMD patients (Fig. 1).[8,21,23,25,26] These represent a loss of posteriorly directed forces due to the selective scarring of the posterobasal portion of the left ventricle that is common in dystrophic myocardium.[27] This myocardial scarring can also extend laterally and produce large Q waves that are most frequently seen in the lateral leads (I, aVL, V6) and, less frequently, in the inferior (II, III, aVF) or anterior leads (V1–V4). Other findings include shortened PR interval, prolonged QTc intervals, and premature atrial and ventricular contractions.[8,24,26–28]

Holter Monitors. Extended monitoring of the cardiac rhythm can provide greater detail of sporadic abnormalities not seen on the brief electrocardiogram. In DMD, Holter monitoring can demonstrate variations in heart rate and associated arrhythmias (Fig. 2). Kirchmann et al. showed that Holter monitoring in DMD demonstrated sinus tachycardia in 26% of patients, deprivation of circadian rhythm in 31% of patients, and reduced heart rate variability in 51% of patients.[29] D'Orsogna et al. described labile abrupt sinus tachycardia in 11 of 18 cases.[23] Yotsukura et al. also found a higher ratio of sympathetic to parasympathetic activity in DMD patients compared with normal controls.[30,31] Similarly, Lanza et al. showed impairment of cardiac autonomic function with an increased ratio of sympathetic activity.[32] These Holter results could reflect disturbances in the cardiac autonomic nervous system due to focal degeneration of the conduction system or adaptation to heart failure in DMD patients.

Holter monitoring can also capture arrhythmias. D'Orsogna et al. reported 4 of 18 DMD patients who developed high-grade ventricular ectopy.[23] Chenard et al. showed that 15% of DMD patients had premature ventricular beats and that 66% of patients who died suddenly had previously documented complex ventricular arrhythmias.[33] Corrado et al. found >6 premature ventricular beats per hour in 32% and ventricular tachycardia in 7% of DMD patients monitored.[34] Kirchmann et al. found premature ventricular beats in 9% of DMD patients.[29] Thus, especially in the presence of symptoms or decreased cardiac function, Holter monitoring can aid in the diagnosis of arrhythmias and direct appropriate medical therapy.

Echocardiography. The "gold standard" of cardiac systolic function evaluation in DMD is shortening fraction (SF) and ejection fraction (EF) using two-dimensional (2D) echocardiography (Fig. 3). Many of the physical attributes of DMD patients, including barrel-shaped chest, increased adiposity of

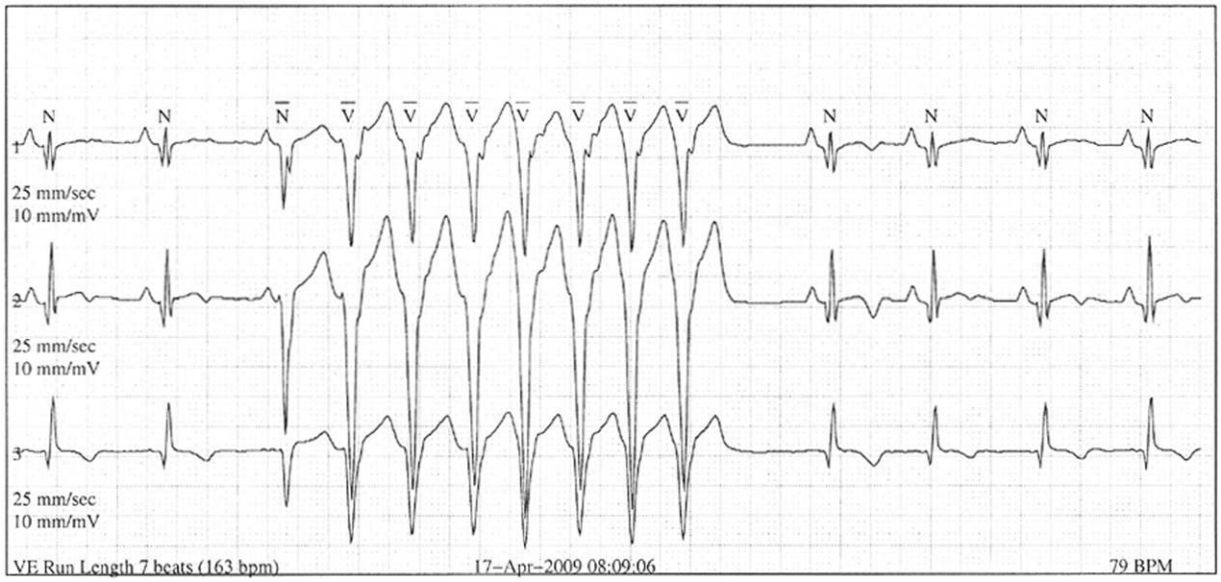

FIGURE 2. Holter monitor tracing of an 18-year-old DMD patient. The tracing shows a non-sustained run of ventricular tachycardia at a rate of approximately 160 beats per minute. The patient was asymptomatic during the recording. N, normal sinus beat; V, abnormal ventricular beat.

chest walls, scoliosis, and seated position, make echocardiography more difficult. Decreased image resolution makes delineation of the endocardial border more difficult and prone to measurement error. These standard measures of cardiac function also become more limited as patients become older. However, echocardiography is the most universally standardized assessment of cardiac function at this time.

As noted, evidence of cardiac disease can be seen on electrocardiogram (ECG) at an early age, long before any decrease in cardiac systolic function is detected by 2D echocardiography. To help diagnose these early changes, different echocardiographic techniques are used. One measurement, the myocardial performance index (MPI), is an assessment of global heart function based on time intervals during the cardiac cycle spent in ejection and isovolumic periods.[35] The MPI is easily measured using Doppler images and was shown to closely relate to the EF.[36] Bahler et al. showed that the calculation of MPI was feasible in DMD and detected abnormalities in 79% of patients when the EF was abnormal in only 40% of these patients.[37] Another measure is Doppler tissue imaging (DTI). DTI is utilized to help detect early changes in cardiac systolic and diastolic function (Fig. 3). DTI does not require good 2D resolution and can provide specific information on myocardial tissue velocities and strain. Giatrakos et al. found decreased tissue velocities in asymptomatic DMD boys with a mean age of 8.8 years. Based on these measures, the investigators correctly predicted poor outcomes with 85% accuracy.[38] Markham et al. found abnormal diastolic indices in DMD patients with normal systolic function compared with controls.[39] Mori et al. showed significantly decreased peak systolic radial strain in the posterior wall compared with controls.[40] Decreased strain was seen more frequently in the outer portion of the posterior wall, a finding consistent with findings of subepicardial fibrosis in heart specimens.[41] Ogata et al. also showed abnormal strain profiles in the posterolateral wall of the left ventricle in DMD patients with normal systolic function.[42] Mertens et al. demonstrated significantly decreased longitudinal and radial tissue velocities in the anterolateral and inferolateral left ventricular walls in DMD patients (mean age 7.9 years) with normal systolic function.[43] These studies demonstrate the presence of myocardial dysfunction prior to the development of decreased systolic function and validate the importance of MPI and DTI as a primary outcome measures for future DMD cardiac studies.

MRI. Due to the imaging difficulties with echocardiography discussed previously, cardiac magnetic resonance (CMR) imaging is being more frequently utilized in DMD patients, providing a sensitive and reliable non-invasive measure of cardiac function. Compared with echocardiography, CMR imaging utilizes different techniques to assess myocardial function. Ashford et al. used CMR tagging to show that DMD patients with normal left ventricular size and function had decreased global and segmental circumferential strain compared with controls.[44] Mavrogeni et al. used CMR to

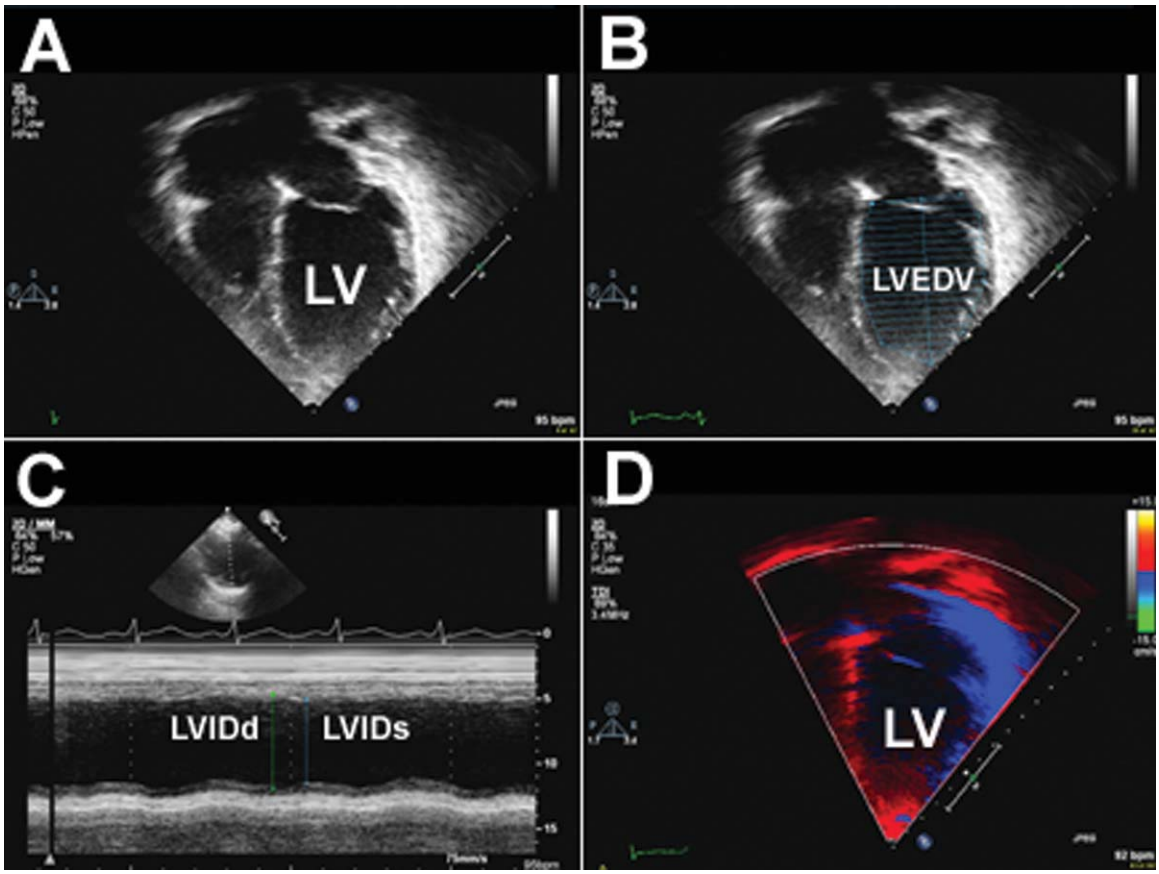

FIGURE 3. Echocardiographic images of a 16-year-old DMD patient with cardiomyopathy. **(A)** Two-dimensional apical four-chamber view of a dilated left ventricle (LV) showing thin and rounded lateral myocardial wall and septum. **(B)** Measurement of the left ventricle end-diastolic volume (LVEDV) in the apical four-chamber view used to calculate ejection fraction. The ejection fraction in this patient was severely decreased at 20% (normal 55–65%). **(C)** M-mode image of the left ventricle showing decreased movement of the interventricular septum at the top of the image and the lateral free wall at the bottom of the image. The left ventricular internal diameter in diastole (LVIDd: green line) and the left ventricular internal diameter in systole (LVIDs: blue line) are measured to derive the shortening fraction (LVIDd − LVIDs / LVIDd). This image shows a severely decreased shortening fraction of 9% (normal 28–40%). **(D)** Color tissue Doppler image of the left ventricle (LV). The coloring of the ventricular myocardium corresponds to myocardial velocities that are measured to evaluate diastolic function and myocardial strain. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

measure T2 relaxation time to study myocardial tissue composition. T2 relaxation times decrease with fibrosis, and the investigators found significantly decreased times in DMD patients >12 years old compared with controls. The T2 times also decreased with age in DMD patients and could be a potential marker of worsening disease.[45] A later study also showed that patients treated with deflazacort showed better preservation of T2 relaxation times in the myocardium and better systolic function compared with untreated younger controls.[46] Hor et al. found that DMD patients with normal EF showed reduced left ventricular myocardial peak circumferential strain when <10 years old compared with controls and this continued to decline with age.[47] Hagenbuch et al. studied serial circumferential strain changes in DMD patients and found significantly decreased strain in all patients over a mean interval of 15.6 months with no significant changes in EF.[48] Thus, strain derived from CMR is another potential early marker of myocardial dysfunction in DMD. It is more sensitive than EF and potentially can be followed longitudinally to monitor changes related to experimental therapies.

Another technique used with CMR is termed late gadolinium enhancement (LGE). Gadolinium-based contrast is given to the patient, and imaging is performed 10–20 minutes thereafter. Areas that are fibrotic retain the contrast due to diminished washout (Fig. 4). Using this technique, Silva et al. showed that midwall and subepicardial fibrosis was present in 7 of 10 muscular dystrophy patients, most commonly in the lateral wall of the left ventricle. Decreased systolic function was only seen in 3 of these patients, and the study found fibrosis in 2 of 4 patients who were <10 years of age.[49] Similar techniques were applied to patients with BMD, and CMR again showed increased cardiac involvement compared with echocardiography.[50] Guillaume et al. presented a case report using CMR

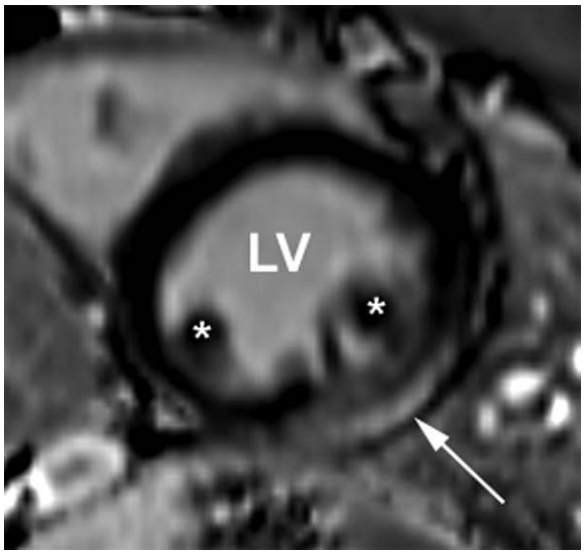

FIGURE 4. Cardiac magnetic resonance (CMR) image utilizing late gadolinium enhancement (LGE) in DMD. The cavity of the left ventricle (LV) is centered, with the papillary muscles present (*). The arrow indicates a bright area of LGE in the subepicardial basal inferior wall that extends into the midwall of the myocardium. The septal and right ventricular myocardiums are spared. This is a typical pattern seen in DMD (image courtesy of Dr. Erik Schelbert, University of Pittsburgh).

and LGE to document an increase in areas of myocardial fibrosis over the course of 1 year.[51] Puchalski et al. observed 74 DMD patients (mean age 13.7 years) and found LGE in older patients (16.6 vs. 13.0 years), and these patients had significantly decreased EFs (24.6% vs. 61.5%). All patients with LGE showed involvement of the basal inferolateral left ventricular free wall in the subepicardial area.[52] Importantly, some patients with LGE were <12.5 years old and had normal EF, indicating that fibrosis occurs prior to the onset of decreased systolic function. Two cases were also reported using CMR to help diagnose myocarditis on DMD patients based on clinical presentation and septal location of LGE.[53,54] These studies demonstrated that CMR, combined with strain and LGE analysis, is more sensitive than echocardiography and will continue to become a more prevalent modality for the primary assessment of cardiac function in DMD patients.

Laboratory Monitoring. B-type natriuretic peptide (BNP) is now used commonly to diagnose and follow heart failure patients.[55] Interestingly, DMD patients do not have the consistency in BNP levels seen in other forms of cardiomyopathy and heart failure. Mori et al. found only late increases in BNP levels in DMD patients associated with significant systolic dysfunction.[56] Kagaya et al. showed that DMD patients had lower BNP levels compared with patients with idiopathic dilated cardiomyopathy who had similar levels of systolic dysfunction.[57] Mohyuddin et al. also found that DMD patients with mild systolic dysfunction had normal BNP levels and only mildly elevated levels when dysfunction worsened.[58] van Bockel et al. showed that N-terminal proBNP levels correlated with decreased systolic function as assessed by radionucleotide scanning, but were overall relatively low.[59] At this time, it is unclear why BNP levels are lower in DMD patients, but possible factors include obesity, physical inactivity, myocardial fibrosis, and potential early use of cardiac medications. These levels may be appropriate for the longitudinal assessment of cardiac function in a single patient, but correlations should not be made with published levels related to other diagnoses.

MEDICAL THERAPY

Prednisone. Based on the recommendations of an international workshop in 2004, daily steroid therapy became the "gold standard" in DMD.[60] These recommendations were based on many studies that showed the benefits of steroids on skeletal and respiratory muscle function.[14,61–63] Studies then began to focus on the cardiac effects. Silversides et al. showed that only 5% of patients treated with deflazacort for ≥3 years had a significantly decreased EF, compared with 58% of untreated patients. They also showed a correlation between preserved cardiac function and improved pulmonary and skeletal muscle function.[64] Markham et al. found that steroid-naive subjects ≤10 years old were 4.4 times more likely to have decreased cardiac function, and those subjects >10 years were 15.2 times more likely to have decreased function. Of interest, patients who had received steroids, but were no longer taking them, showed normal cardiac function and no differences compared with patients who continued to receive steroids.[65] Biggar et al. showed that 59% of untreated DMD patients developed decreased cardiac function by 18 years of age, compared with 10% of steroid-treated patients.[63] Houde et al. found that deflazacort treatment preserved cardiac function in DMD patients over an 8-year follow-up study. Treated patients had improved systolic function and a decreased incidence of dilated cardiomyopathy (32% vs. 58%) compared with younger untreated patients.[66] Markham et al. reported that 93% of steroid-treated DMD children maintained normal cardiac function compared with only 53% of untreated children. These studies support the idea that DMD patients treated with steroids prior to the onset of cardiac dysfunction show slower progression of heart disease. The study by Markham et al. also questioned whether there is an early therapeutic window for obtaining the beneficial effects of steroid therapy in cardiac muscle.

However, questions still remain regarding the best type of steroid, the age of therapy initiation, the dosing schedule, and the duration of therapy. Further studies are needed to address these questions and to utilize improved cardiac outcome measures.

Currently there are a few animal studies that question the benefits of steroids. Bauer et al. showed that prednisone delivered via drinking water led to increased left ventricular dilation, decreased diastolic function, and increased cardiac fibrosis.[67] Guerron et al. used a subcutaneous prednisone pellet to deliver continuous drug at a dose of 1 mg/kg/day. They found significantly decreased cardiac function and increased cardiac fibrosis in prednisone-treated *mdx* mice.[68] However, in the previously mentioned clinical studies, steroid use in DMD patients was not associated with any decreases in cardiac function. These two animal studies used more continuous delivery methods that may be more deleterious than the single-dose therapies used clinically. Recent evidence from our laboratory showed that continuous steroids can disrupt cell cycling and cytokine signaling, leading to increased inflammation and fibrosis (E. Hoffman, personal communication). Further studies in animal models should more closely replicate clinical dosing schedules to better assess any deleterious effects. Based on the current literature, evidence favors the beneficial effects of single-dose steroids on cardiac function in DMD patients.

ACE Inhibitors and β-Blockers. The decreased cardiac function seen in cardiomyopathies stimulates the renin–angiotensin system and leads to the release of angiotensin II (ATII). Among its many actions, ATII is a potent stimulator of transforming growth factor-β (TGF-β), which promotes fibrosis.[69] Angiotensin-converting enzyme inhibitors (ACEIs) modulate the production of ATII by preventing the conversion from angiotensin I to ATII and may benefit cardiac function by limiting the amount of fibrosis and scarring within the myocardium. These drugs are widely used and recommended by the American Heart Association for the prevention and treatment of heart failure.[70]

Accordingly, ACEIs were studied in DMD-related cardiomyopathy. Ishikawa et al. reported a reduction in neuroendocrine activity and left ventricular dilation in DMD patients taking ACEIs and β-blockers.[71] Duboc et al. studied 57 children with DMD, aged 9.3–13 years, with normal cardiac function (EF >55%). In the initial phase, 27 children were started on the ACEI perindopril (2–4 mg/day), and 29 children received placebo for 3 years. After this period, all patients ($n = 51$) received perindopril for 2 years. There were no significant differences at the start or end of the initial 3 years. However, at the completion of the second phase, 8 patients in the initial untreated group developed an EF of <45% compared with 1 patient in the treated group.[72] The same investigators published results after 10 years of follow-up. Although all patients started with normal cardiac function, 93% of the initial treated group were alive vs. only 66% of the untreated group.[73] The investigators stated that early treatment delayed the onset and progression of left ventricular dysfunction and led to lower mortality in DMD. Ramaciotti et al. also showed a benefit from ACEI treatment. In a retrospective analysis of 50 patients with DMD aged 10–20 years, 10 of 27 patients with systolic dysfunction returned to normal function after treatment with the ACEI enalapril.[74] Jefferies et al. followed DMD and BMD patients with a mean age of 12.9 years and 13.7 years, respectively. After the first abnormal echocardiogram (EF <55%), patients were started on an ACEI and, if no improvement was seen at 3 months, β-blockers were added. ACEI was the single therapy in 42% of patients, and combination therapy was required in 58% of patients. ACEI or combination therapy improved cardiac function in 27 of 29 patients.[17] Kajimoto et al. showed that combination therapy of carvedilol and an ACEI for 2 years resulted in a significant increase in systolic function in a mixed muscular dystrophy cohort.[71,75] The β-blocker carvedilol was studied by Rhodes et al. in DMD patients aged 14–46 years with a dilated cardiomyopathy and EF <50%. Carvedilol was administered for 6 months and was associated with a small but statistically significant improvement in CMR-derived EF (41–43%). Carvedilol also decreased the incidence of ventricular tachycardia seen in 2 patients.[76]

ACEIs are becoming the primary therapy for cardiovascular disease in DMD. Although all of these patients will develop some degree of cardiomyopathy, additional studies are still required to better understand the benefits of early initiation of cardiac "preventive" therapy with ACEIs and β-blockers and any potential interactions with concomitant steroid therapy. Current recommendations continue to advocate the use of ACEI therapy at the first signs of decreased cardiac function.

MONITORING

The routine monitoring of cardiovascular disease in muscular dystrophies is very important. As noninvasive methods for the quantification of cardiac function improve, certain treatments may begin at earlier ages. Two committees have recommended general guidelines for the routine follow-up of cardiovascular disease in muscular dystrophies: the American Academy of Pediatrics Section on Cardiology and Cardiac Surgery, and the 107th ENMC

International Workshop on the Management of Cardiac Involvement in Muscular Dystrophy and Myotonic Dystrophy.[77,78] A complete initial evaluation should be performed for DMD patients at the time of diagnosis. This evaluation should include a history and physical examination, ECG, and echocardiogram. Consideration should be given to further testing, including Holter monitoring and MRI (especially if the patient has poor imaging on transthoracic echocardiography). For DMD, patients should have a complete cardiac evaluation every 1–2 years up to the age of 10 years, and then evaluations should occur yearly.[29] Evaluations should also be performed before any scheduled surgery. Once cardiac disease is identified, follow-up is dictated by the type and severity of cardiac disease. Also, evaluation with more sophisticated tools for detection of preclinical abnormalities at tertiary care centers is recommended when available.

FUTURE THERAPIES

Poloxamer 188. Poloxamer 188 (P188) is a nonionic triblock copolymer, poly(ethylene oxide)$_{80}$-poly(propylene oxide)$_{27}$-poly(ethylene oxide)$_{80}$. It is known to insert into artificial lipid monolayers and repair damaged biological membranes. P188 was shown to stabilize red blood cell membranes in sickle cell disease.[79] Based on these properties, Yasuda et al. studied P188 in the dystrophin-deficient *mdx* mouse heart and showed that administration of P188 during dobutamine infusion prevented the development of acute cardiac failure.[80] Townsend et al. reported that a chronic 8-week infusion of P188 in Golden Retriever muscular dystrophy (GRMD) dogs showed significantly decreased cardiac fibrosis and prevented ventricular dilation.[81] Based on these animal studies, P188 could become an important acute therapy in DMD. Intravenous P188 could provide immediate benefits by preventing cardiac damage in times of increased stress, including orthopedic surgery, respiratory failure, and acutely decompensated heart failure.

Losartan. Just as ACEIs blunt the renin–angiotensin–aldosterone axis, losartan, an ATII-type 1 receptor blocker, also modulates ATII signaling. Based on this action, Cohn et al. studied the effects of losartan in *mdx* mice and showed significantly decreased fibrosis and muscle fiber diameters in the diaphragm. Losartan also restored *in vitro* force frequency in the *mdx* extensor digitorum longus to wild-type levels and improved hindlimb grip strength in treated mice.[82] Matsuhisa et al. looked at the effects of losartan in BIO14.6 cardiomyopathic hamsters and found decreased ventricular dilation, myocardial fibrosis and cardiac dysfunction by inhibiting oxidative stress.[83] In our laboratory we recently showed decreased myocardial fibrosis and preservation of cardiac function in *mdx* mice treated with losartan over a 6-month period.[84] Based on these findings, it is possible that losartan could decrease both skeletal and cardiac muscle fibrosis and preserve skeletal muscle strength and cardiac function in DMD patients. Clinical studies using losartan are currently in progress.

Idebenone. Idebenone, a synthetic analog of coenzyme Q10, is an antioxidant medication shown to improve mitochondrial respiratory chain function and cellular energy production. Clinical studies in Friedreich ataxia showed beneficial effects on cardiac function.[85] Buyse et al. treated *mdx* mice over 9 months and found that it prevented cardiac diastolic dysfunction, prevented dobutamine-induced acute cardiac failure, and decreased cardiac inflammation and fibrosis.[86] Idebenone-treated *mdx* mice also demonstrated increased voluntary running at faster rates for longer distances compared with untreated *mdx* mice. A clinical trial was recently completed studying the effects of idebenone in DMD patients with cardiac dysfunction.

Gene Therapy. Due to the lack of specific medical therapies for DMD at this time, gene therapy offers the promise of a cure by replacing the mutated dystrophin gene in all muscle tissues. However, the development of gene therapy techniques has faced multiple challenges. First, the dystrophin gene (2.1 million basepairs) is too large to transfer via known vector systems. This led to the development of mini-/micro-dystrophin, smaller, partially functional proteins with portions of the rod and C-terminal domain removed. Yue et al. showed improved sarcolemmal integrity of the hearts of *mdx* mice with microdystrophin gene therapy using recombinant adeno-associated virus (rAAV).[87] Gregorevic et al. also reported cardiac expression of micro-dystrophin in *mdx* mice using rAAV vectors.[88] Townsend et al. showed that transduction of micro-dystrophin in the *mdx* heart using rAAV prevented acute cardiac pump failure during dobutamine stress.[89] Bostick et al. showed that cardiac expression of mini-dystrophin on 20–22-month-old *mdx* mice decreased cardiac fibrosis and significantly improved, but did not normalize, all cardiac parameters.[90] These animal studies continue to be promising, but significant concerns were recently published. Mendell et al. treated the skeletal muscle of DMD patients with rAAV-mediated mini-dystrophin and reported the development of dystrophin-reactive T cells.[91] These results raise concerns for serious immune-mediated limitations not only to the vectors, but to the dystrophin protein itself.[92]

Stem Cell Transplant. Initial cell-based therapies in DMD focused on myoblast transplantation. Multiple studies showed limited or no expression of dystrophin after direct myoblast injection.[93–97] Due to the limited success, research expanded to include stem cells that were myogenic precursors. Much of this research is beyond the scope of this review, but several sources of potential stem cells were identified, including bone marrow, satellite cells, muscle, and blood-derived stem cells.[98] There are multiple case reports of cord blood transplantation with limited results.[99–102] Just as in gene therapy, immune responses also became a concern.[103] Due to this, the field focused on autologous stem cell populations.[104] In a major breakthrough, Takahashi and Yamanaka first reported the generation of induced pluripotent stem cells (iPS) in mice.[105] Their concept was to reprogram adult cells back to a state of pluripotency. This process would allow for the generation of patient-specific stem cells that could be driven toward a myogenic lineage. There are still significant hurdles, but this methodology could help avoid immunological barriers and future political battles. Most current stem cell therapy research in cardiac muscle is directed toward the treatment of myocardial infarction and heart failure. One apparent mechanism involved in cardiac stem cell therapy is the local secretion of growth factors and cytokines, both by the stem cells and host tissues.[106] It is not known how this signaling may differ in a dystrophin-deficient cell, so any benefits of cardiac stem cell therapy in an ischemic environment may not be applicable to DMD. Significant further research is required in both fields before stem cell therapy becomes a viable treatment strategy.

Exon Skipping. The basis of exon-skipping therapy is to use splice-switching oligonucleotides to bypass the mutated exon with a stop codon and continue to translate a smaller, truncated dystrophin protein. The goal is to produce a BMD phenotype with a partially functional dystrophin protein expressed in muscle tissues. Studies in human skeletal muscle demonstrate proof of this concept.[107,108] However, animal studies showed limited expression of dystrophin in cardiac tissue using initial oligonucleotide formulations.[109–111] Further studies conjugated the phosphorodiamidate morpholino oligomer (PMO) with a cell-penetrating, arginine-rich peptide, creating a novel PPMO, and demonstrated cardiac dystrophin expression and functional improvements.[112,113] Most recently, Jearawiriyapaisarn et al. gave *mdx* mice PPMOs and restored cardiac dystrophin expression, decreased serum creatine kinase, and improved cardiac hypertrophy and diastolic function for up to 7 months after the start of treatment.[114] Exon skipping has produced the most exciting results to date, and further systemic testing of PPMOs is necessary in animal models before initiation of human clinical trials.

As these treatments continue to develop, the same concern arises as discussed previously in relation to steroid therapy and exercise. Improvements in skeletal and not cardiac muscle could become a limiting factor in relation to gene, stem cell, or exon-skipping therapy. For example, Townsend et al. studied *mdx* mice with transgene production of mini-dystrophin in skeletal and diaphragm muscle only and showed normal skeletal muscle strength and increased voluntary wheel running. However, after 4–5 months, the hearts of transgene mice showed increased left ventricular dilation and significant decline in systolic function.[13] This type of result continues to raise ethical questions. Would correction of skeletal muscle function without cardiac muscle correction lead to increased incidence of cardiomyopathy? As initial studies will no doubt focus solely on skeletal muscle, the true effects on cardiac muscle in DMD patients may not be known initially. Without any better cardiac therapies at this time, the incidence of cardiomyopathy could increase from skeletal muscle therapies. Cardiac transplantation cannot be considered a viable solution to treat DMD patients who walk into the intensive care unit because of successful skeletal muscle therapies.

CONCLUSIONS

Cardiomyopathy in DMD is becoming more prevalent due to improved treatments for the skeletal and respiratory systems. Current cardiomyopathy therapies, although not specific to DMD, benefit cardiac function in DMD and improve outcomes. Steroid therapy appears to slow the progression of cardiomyopathy at a young age. Further research is needed to define the role of ACEIs and/or β-blockers in preclinical therapy. Use of echocardiography and CMR imaging modalities has led to preclinical outcome measures that make this feasible. As knowledge of the specific mechanisms involved in DMD muscle cell death broaden, new targeted therapeutic strategies can be developed to help further prevent and slow the progression of cardiomyopathy. Also, while new genetic modifying therapies are being developed, it must be done with the foresight that cardiac and skeletal muscle be treated equally. Continued development of novel cardiac therapies will help improve the quality and duration of life in DMD patients.

REFERENCES

1. Eagle M, Baudouin SV, Chandler C, Giddings DR, Bullock R, Bushby K. Survival in Duchenne muscular dystrophy: improvements

in life expectancy since 1967 and the impact of home nocturnal ventilation. Neuromuscul Disord 2002;12:926–929.
2. Eagle M, Bourke J, Bullock R, Gibson M, Mehta J, Giddings D, et al. Managing Duchenne muscular dystrophy—the additive effect of spinal surgery and home nocturnal ventilation in improving survival. Neuromuscul Disord 2007;17:470–475.
3. Williams IA, Allen DG. Intracellular calcium handling in ventricular myocytes from *mdx* mice. Am J Physiol Heart Circ Physiol 2007;292: H846–855.
4. Woolf PJ, Lu S, Cornford-Nairn R, Watson M, Xiao XH, Holroyd SM, et al. Alterations in dihydropyridine receptors in dystrophin-deficient cardiac muscle. Am J Physiol Heart Circ Physiol 2006;290: H2439–H2445.
5. Whitehead NP, Yeung EW, Allen DG. Muscle damage in *mdx* (dystrophic) mice: role of calcium and reactive oxygen species. Clin Exp Pharmacol Physiol 2006;33:657–662.
6. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation 2006;113:1807–1816.
7. Piccolo G, Azan G, Tonin P, Arbustini E, Gavazzi A, Banfi P, et al. Dilated cardiomyopathy requiring cardiac transplantation as initial manifestation of Xp21 Becker type muscular dystrophy. Neuromuscul Disord 1994;4:143–146.
8. Saito M, Kawai H, Akaike M, Adachi K, Nishida Y, Saito S. Cardiac dysfunction with Becker muscular dystrophy. Am Heart J 1996;132: 642–647.
9. Berko BA, Swift M. X-linked dilated cardiomyopathy. N Engl J Med 1987;316:1186–1191.
10. Towbin JA, Hejtmancik JF, Brink P, Gelb B, Zhu XM, Chamberlain JS, et al. X-linked dilated cardiomyopathy. Molecular genetic evidence of linkage to the Duchenne muscular dystrophy (dystrophin) gene at the Xp21 locus. Circulation 1993;87:1854–1865.
11. Costas JM, Nye DJ, Henley JB, Plochocki JH. Voluntary exercise induces structural remodeling in the hearts of dystrophin-deficient mice. Muscle Nerve 2010;42:881–885.
12. Nakamura A, Yoshida K, Takeda S, Dohi N, Ikeda S. Progression of dystrophic features and activation of mitogen-activated protein kinases and calcineurin by physical exercise, in hearts of *mdx* mice. FEBS Lett 2002;520:18–24.
13. Townsend D, Yasuda S, Li S, Chamberlain JS, Metzger JM. Emergent dilated cardiomyopathy caused by targeted repair of dystrophic skeletal muscle. Mol Ther 2008;16:832–835.
14. DeSilva S, Drachman DB, Mellits D, Kuncl RW. Prednisone treatment in Duchenne muscular dystrophy. Long-term benefit. Arch Neurol 1987;44:818–822.
15. Kaspar RW, Allen HD, Ray WC, Alvarez CE, Kissel JT, Pestronk A, et al. Analysis of dystrophin deletion mutations predicts age of cardiomyopathy onset in becker muscular dystrophy. Circ Cardiovasc Genet 2009;2:544–551.
16. Nigro G, Politano L, Nigro V, Petretta VR, Comi LI. Mutation of dystrophin gene and cardiomyopathy. Neuromuscul Disord 1994;4: 371–379.
17. Jefferies JL, Eidem BW, Belmont JW, Craigen WJ, Ware SM, Fernbach SD, et al. Genetic predictors and remodeling of dilated cardiomyopathy in muscular dystrophy. Circulation 2005;112:2799–2804.
18. Mavrogeni S, Papavasiliou A, Spargias K, Constandoulakis P, Papadopoulos G, Karanasios E, et al. Myocardial inflammation in Duchenne muscular dystrophy as a precipitating factor for heart failure: a prospective study. BMC Neurol 2010;10:33.
19. Xiong D, Lee GH, Badorff C, Dorner A, Lee S, Wolf P, et al. Dystrophin deficiency markedly increases enterovirus-induced cardiomyopathy: a genetic predisposition to viral heart disease. Nat Med 2002;8:872–877.
20. Nigro G, Comi LI, Politano L, Bain RJ. The incidence and evolution of cardiomyopathy in Duchenne muscular dystrophy. Int J Cardiol 1990;26:271–277.
21. Gilroy J, Cahalan JL, Berman R, Newman M. Cardiac and pulmonary complications in Duchenne's progressive muscular dystrophy. Circulation 1963;27:484–493.
22. Oguz D, Olgunturk R, Gucuyener K, Acikgoz GV, Tunaoglu FS. A comparison between MUGA and echocardiography in patients with muscular dystrophy in the early detection of cardiac involvement. Pediatr Cardiol 1998;19:150–154.
23. D'Orsogna L, O'Shea JP, Miller G. Cardiomyopathy of Duchenne muscular dystrophy. Pediatr Cardiol 1988;9:205–213.
24. Bhattacharyya KB, Basu N, Ray TN, Maity B. Profile of electrocardiographic changes in Duchenne muscular dystrophy. J Indian Med Assoc 1997;95:40–42, 47.
25. Melacini P, Fanin M, Danieli GA, Villanova C, Martinello F, Miorin M, et al. Myocardial involvement is very frequent among patients affected with subclinical Becker's muscular dystrophy. Circulation 1996;94:3168–3175.
26. Steare SE, Dubowitz V, Benatar A. Subclinical cardiomyopathy in Becker muscular dystrophy. Br Heart J 1992;68:304–308.
27. Perloff JK, Roberts WC, de Leon AC Jr, O'Doherty D. The distinctive electrocardiogram of Duchenne's progressive muscular dystrophy. An electrocardiographic–pathologic correlative study. Am J Med 1967;42:179–188.
28. Ishikawa K. Cardiac involvement in progressive muscular dystrophy of the Duchenne type. Jpn Heart J 1997;38:163–180.
29. Kirchmann C, Kececioglu D, Korinthenberg R, Dittrich S. Echocardiographic and electrocardiographic findings of cardiomyopathy in Duchenne and Becker–Kiener muscular dystrophies. Pediatr Cardiol 2005;26:66–72.
30. Yotsukura M, Fujii K, Katayama A, Tomono Y, Ando H, Sakata K, et al. Nine-year follow-up study of heart rate variability in patients with Duchenne-type progressive muscular dystrophy. Am Heart J 1998;136:289–296.
31. Yotsukura M, Sasaki K, Kachi E, Sasaki A, Ishihara T, Ishikawa K. Circadian rhythm and variability of heart rate in Duchenne-type progressive muscular dystrophy. Am J Cardiol 1995;76:947–951.
32. Lanza GA, Dello Russo A, Giglio V, De Luca L, Messano L, Santini C, et al. Impairment of cardiac autonomic function in patients with Duchenne muscular dystrophy: relationship to myocardial and respiratory function. Am Heart J 2001;141:808–812.
33. Chenard AA, Becane HM, Tertrain F, de Kermadec JM, Weiss YA. Ventricular arrhythmia in Duchenne muscular dystrophy: prevalence, significance and prognosis. Neuromuscul Disord 1993;3:201–206.
34. Corrado G, Lissoni A, Beretta S, Terenghi L, Tadeo G, Foglia-Manzillo G, et al. Prognostic value of electrocardiograms, ventricular late potentials, ventricular arrhythmias, and left ventricular systolic dysfunction in patients with Duchenne muscular dystrophy. Am J Cardiol 2002;89:838–841.
35. Tei C, Ling LH, Hodge DO, Bailey KR, Oh JK, Rodeheffer RJ, et al. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function—a study in normals and dilated cardiomyopathy. J Cardiol 1995;26:357–366.
36. LaCorte JC, Cabreriza SE, Rabkin DG, Printz BF, Coku L, Weinberg A, et al. Correlation of the Tei index with invasive measurements of ventricular function in a porcine model. J Am Soc Echocardiogr 2003;16:442–447.
37. Bahler RC, Mohyuddin T, Finkelhor RS, Jacobs IB. Contribution of Doppler tissue imaging and myocardial performance index to assessment of left ventricular function in patients with Duchenne's muscular dystrophy. J Am Soc Echocardiogr 2005;18:666–673.
38. Giatrakos N, Kinali M, Stephens D, Dawson D, Muntoni F, Nihoyannopoulos P. Cardiac tissue velocities and strain rate in the early detection of myocardial dysfunction of asymptomatic boys with Duchenne's muscular dystrophy: relationship to clinical outcome. Heart 2006;92:840–842.
39. Markham LW, Michelfelder EC, Border WL, Khoury PR, Spicer RL, Wong BL, et al. Abnormalities of diastolic function precede dilated cardiomyopathy associated with Duchenne muscular dystrophy. J Am Soc Echocardiogr 2006;19:865–871.
40. Mori K, Hayabuchi Y, Inoue M, Suzuki M, Sakata M, Nakagawa R, et al. Myocardial strain imaging for early detection of cardiac involvement in patients with Duchenne's progressive muscular dystrophy. Echocardiography 2007;24:598–608.
41. Frankel KA, Rosser RJ. The pathology of the heart in progressive muscular dystrophy: epimyocardial fibrosis. Hum Pathol 1976;7: 375–386.
42. Ogata H, Nakatani S, Ishikawa Y, Negishi A, Kobayashi M, Minami R. Myocardial strain changes in Duchenne muscular dystrophy without overt cardiomyopathy. Int J Cardiol 2007;115:190–195.
43. Mertens L, Ganame J, Claus P, Goemans N, Thijs D, Eyskens B, et al. Early regional myocardial dysfunction in young patients with Duchenne muscular dystrophy. J Am Soc Echocardiogr 2008;21:1049–1054.
44. Ashford MW Jr, Liu W, Lin SJ, Abraszewski P, Caruthers SD, Connolly AM, et al. Occult cardiac contractile dysfunction in dystrophin-deficient children revealed by cardiac magnetic resonance strain imaging. Circulation 2005;112:2462–2467.
45. Mavrogeni S, Tzelepis GE, Athanasopoulos G, Maounis T, Douskou M, Papavasiliou A, et al. Cardiac and sternocleidomastoid muscle involvement in Duchenne muscular dystrophy: an MRI study. Chest 2005;127:143–148.
46. Mavrogeni S, Papavasiliou A, Douskou M, Kolovou G, Papadopoulou E, Cokkinos DV. Effect of deflazacort on cardiac and sternocleidomastoid muscles in Duchenne muscular dystrophy: a magnetic resonance imaging study. Eur J Paediatr Neurol 2009;13:34–40.
47. Hor KN, Wansapura J, Markham LW, Mazur W, Cripe LH, Fleck R, et al. Circumferential strain analysis identifies strata of cardiomyopathy in Duchenne muscular dystrophy: a cardiac magnetic resonance tagging study. J Am Coll Cardiol 2009;53:1204–1210.
48. Hagenbuch SC, Gottliebson WM, Wansapura J, Mazur W, Fleck R, Benson DW, et al. Detection of progressive cardiac dysfunction by

serial evaluation of circumferential strain in patients with Duchenne muscular dystrophy. Am J Cardiol 2010;105:1451–1455.
49. Silva MC, Meira ZM, Gurgel Giannetti J, da Silva MM, Campos AF, Barbosa Mde M, et al. Myocardial delayed enhancement by magnetic resonance imaging in patients with muscular dystrophy. J Am Coll Cardiol 2007;49:1874–1879.
50. Yilmaz A, Gdynia HJ, Baccouche H, Mahrholdt H, Meinhardt G, Basso C, et al. Cardiac involvement in patients with Becker muscular dystrophy: new diagnostic and pathophysiological insights by a CMR approach. J Cardiovasc Magn Reson 2008;10:50.
51. Guillaume MD, Phoon CK, Chun AJ, Srichai MB. Delayed enhancement cardiac magnetic resonance imaging in a patient with Duchenne muscular dystrophy. Tex Heart Inst J 2008;35:367–368.
52. Puchalski MD, Williams RV, Askovich B, Sower CT, Hor KH, Su JT, et al. Late gadolinium enhancement: precursor to cardiomyopathy in Duchenne muscular dystrophy? Int J Cardiovasc Imaging 2009; 25:57–63.
53. Iriart X, Vogels G, Lederlin M. Combined computed tomography angiography and cardiac magnetic resonance imaging for diagnosis of acute myocarditis in a child with Duchenne myopathy. Pediatr Cardiol 2009;30:1030–1031.
54. Mavrogeni S, Papavassiliou A, Cokkinos DV. Myocarditis in a patient with Duchenne muscular dystrophy detected by cardiovascular magnetic resonance and cardiac biopsy. Int J Cardiol 2009;132: e123–124.
55. Maisel AS, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002;347:161–167.
56. Mori K, Manabe T, Nii M, Hayabuchi Y, Kuroda Y, Tatara K. Plasma levels of natriuretic peptide and echocardiographic parameters in patients with Duchenne's progressive muscular dystrophy. Pediatr Cardiol 2002;23:160–166.
57. Demachi J, Kagaya Y, Watanabe J, Sakuma M, Ikeda J, Kakuta Y, et al. Characteristics of the increase in plasma brain natriuretic peptide level in left ventricular systolic dysfunction, associated with muscular dystrophy in comparison with idiopathic dilated cardiomyopathy. Neuromuscul Disord 2004;14:732–739.
58. Mohyuddin T, Jacobs IB, Bahler RC. B-type natriuretic peptide and cardiac dysfunction in Duchenne muscular dystrophy. Int J Cardiol 2007;119:389–391.
59. van Bockel EA, Lind JS, Zijlstra JG, Wijkstra PJ, Meijer PM, van den Berg MP, et al. Cardiac assessment of patients with late stage Duchenne muscular dystrophy. Neth Heart J 2009;17:232–237.
60. Bushby K, Muntoni F, Urtizberea A, Hughes R, Griggs R. Report on the 124th ENMC International Workshop. Treatment of Duchenne muscular dystrophy; defining the gold standards of management in the use of corticosteroids, 2–4 April 2004, Naarden, The Netherlands. Neuromuscul Disord 2004;14:526–534.
61. Biggar WD, Gingras M, Fehlings DL, Harris VA, Steele CA. Deflazacort treatment of Duchenne muscular dystrophy. J Pediatr 2001; 138:45–50.
62. Balaban B, Matthews DJ, Clayton GH, Carry T. Corticosteroid treatment and functional improvement in Duchenne muscular dystrophy: long-term effect. Am J Phys Med Rehabil 2005;84:843–850.
63. Biggar WD, Harris VA, Eliasoph L, Alman B. Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. Neuromuscul Disord 2006;16:249–255.
64. Silversides CK, Webb GD, Harris VA, Biggar DW. Effects of deflazacort on left ventricular function in patients with Duchenne muscular dystrophy. Am J Cardiol 2003;91:769–772.
65. Markham LW, Spicer RL, Khoury PR, Wong BL, Mathews KD, Cripe LH. Steroid therapy and cardiac function in Duchenne muscular dystrophy. Pediatr Cardiol 2005;26:768–771.
66. Houde S, Filiatrault M, Fournier A, Dube J, D'Arcy S, Berube D, et al. Deflazacort use in Duchenne muscular dystrophy: an 8-year follow-up. Pediatr Neurol 2008;38:200–206.
67. Bauer R, Straub V, Blain A, Bushby K, MacGowan GA. Contrasting effects of steroids and angiotensin-converting-enzyme inhibitors in a mouse model of dystrophin-deficient cardiomyopathy. Eur J Heart Fail 2009;11:463–471.
68. Guerron AD, Rawat R, Sali A, Spurney CF, Pistilli E, Cha HJ, et al. Functional and molecular effects of arginine butyrate and prednisone on muscle and heart in the *mdx* mouse model of Duchenne muscular dystrophy. PLoS One 2010;5:e11220.
69. Khan R, Sheppard R. Fibrosis in heart disease: understanding the role of transforming growth factor-beta in cardiomyopathy, valvular disease and arrhythmia. Immunology 2006;118:10–24.
70. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, et al. ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (writing committee to update the 2001 guidelines for the evaluation and management of heart failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. Circulation 2005;112:e154–235.
71. Ishikawa Y, Bach JR, Minami R. Cardioprotection for Duchenne's muscular dystrophy. Am Heart J 1999;137:895–902.
72. Duboc D, Meune C, Lerebours G, Devaux JY, Vaksmann G, Becane HM. Effect of perindopril on the onset and progression of left ventricular dysfunction in Duchenne muscular dystrophy. J Am Coll Cardiol 2005;45:855–857.
73. Duboc D, Meune C, Pierre B, Wahbi K, Eymard B, Toutain A, et al. Perindopril preventive treatment on mortality in Duchenne muscular dystrophy: 10 years' follow-up. Am Heart J 2007;154:596–602.
74. Ramaciotti C, Heistein LC, Coursey M, Lemler MS, Eapen RS, Iannaccone ST, et al. Left ventricular function and response to enalapril in patients with Duchenne muscular dystrophy during the second decade of life. Am J Cardiol 2006;98:825–827.
75. Kajimoto H, Ishigaki K, Okumura K, Tomimatsu H, Nakazawa M, Saito K, et al. Beta-blocker therapy for cardiac dysfunction in patients with muscular dystrophy. Circ J 2006;70:991–994.
76. Rhodes J, Margossian R, Darras BT, Colan SD, Jenkins KJ, Geva T, et al. Safety and efficacy of carvedilol therapy for patients with dilated cardiomyopathy secondary to muscular dystrophy. Pediatr Cardiol 2008;29:343–351.
77. Cardiovascular health supervision for individuals affected by Duchenne or Becker muscular dystrophy. Pediatrics 2005;116:1569–1573.
78. Bushby K, Muntoni F, Bourke JP. 107th ENMC International Workshop: The Management of Cardiac Involvement in Muscular Dystrophy and Myotonic Dystrophy, 7th–9th June 2002, Naarden, The Netherlands. Neuromuscul Disord 2003;13:166–172.
79. Ballas SK, Files B, Luchtman-Jones L, Benjamin L, Swerdlow P, Hilliard L, et al. Safety of purified poloxamer 188 in sickle cell disease: phase I study of a non-ionic surfactant in the management of acute chest syndrome. Hemoglobin 2004;28:85–102.
80. Yasuda S, Townsend D, Michele DE, Favre EG, Day SM, Metzger JM. Dystrophic heart failure blocked by membrane sealant poloxamer. Nature 2005;436:1025–1029.
81. Townsend D, Turner I, Yasuda S, Martindale J, Davis J, Shillingford M, et al. Chronic administration of membrane sealant prevents severe cardiac injury and ventricular dilatation in dystrophic dogs. J Clin Invest 2010;120:1140–1150.
82. Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC, Lisi MT, et al. Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states. Nat Med 2007;13:204–210.
83. Matsuhisa S, Otani H, Okazaki T, Yamashita K, Akita Y, Sato D, et al. N-acetylcysteine abolishes the protective effect of losartan against left ventricular remodeling in cardiomyopathy hamster. Antioxid Redox Signal 2008;10:1999–2008.
84. Spurney CF, Sali A, Guerron AD, Iantorno M, Yu Q, Gordish-Dressman H, et al. Losartan decreases cardiac muscle fibrosis and improves cardiac function in dystrophin-deficient *mdx* mice. J Cardiovasc Pharmacol Ther 2011;16:87–95.
85. Hausse AO, Aggoun Y, Bonnet D, Sidi D, Munnich A, Rotig A, et al. Idebenone and reduced cardiac hypertrophy in Friedreich's ataxia. Heart 2002;87:346–349.
86. Buyse GM, van der Mieren G, Erb M, D'Hooge J, Herijgers P, Verbeken E, et al. Long-term blinded placebo-controlled study of SNT-MC17/idebenone in the dystrophin deficient *mdx* mouse: cardiac protection and improved exercise performance. Eur Heart J 2009; 30:116–124.
87. Yue Y, Li Z, Harper SQ, Davisson RL, Chamberlain JS, Duan D. Microdystrophin gene therapy of cardiomyopathy restores dystrophin–glycoprotein complex and improves sarcolemma integrity in the *mdx* mouse heart. Circulation 2003;108:1626–1632.
88. Gregorevic P, Allen JM, Minami E, Blankinship MJ, Haraguchi M, Meuse L, et al. rAAV6-microdystrophin preserves muscle function and extends lifespan in severely dystrophic mice. Nat Med 2006;12: 787–789.
89. Townsend D, Blankinship MJ, Allen JM, Gregorevic P, Chamberlain JS, Metzger JM. Systemic administration of micro-dystrophin restores cardiac geometry and prevents dobutamine-induced cardiac pump failure. Mol Ther 2007;15:1086–1092.
90. Bostick B, Yue Y, Long C, Marschalk N, Fine DM, Chen J, Duan D. Cardiac expression of a mini-dystrophin that normalizes skeletal muscle force only partially restores heart function in aged *Mdx* mice. Mol Ther 2009;17:253–261.
91. Mendell JR, Rodino-Klapac LR, Malik V. Molecular therapeutic strategies targeting Duchenne muscular dystrophy. J Child Neurol 2010;25:1145–1148.
92. Malik V, Rodino-Klapac LR, Viollet L, Wall C, King W, Al-Dahhak R, et al. Gentamicin-induced readthrough of stop codons in Duchenne muscular dystrophy. Ann Neurol 2010;67:771–780.
93. Gussoni E, Blau HM, Kunkel LM. The fate of individual myoblasts after transplantation into muscles of DMD patients. Nat Med 1997; 3:970–977.

94. Gussoni E, Pavlath GK, Lanctot AM, Sharma KR, Miller RG, Steinman L, et al. Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. Nature 1992;356:435–438.
95. Miller RG, Sharma KR, Pavlath GK, Gussoni E, Mynhier M, Lanctot AM, et al. Myoblast implantation in Duchenne muscular dystrophy: the San Francisco study. Muscle Nerve 1997;20:469–478.
96. Morandi L, Bernasconi P, Gebbia M, Mora M, Crosti F, Mantegazza R, et al. Lack of mRNA and dystrophin expression in DMD patients three months after myoblast transfer. Neuromuscul Disord 1995;5:291–295.
97. Mendell JR, Kissel JT, Amato AA, King W, Signore L, Prior TW, et al. Myoblast transfer in the treatment of Duchenne's muscular dystrophy. N Engl J Med 1995;333:832–838.
98. Farini A, Razini P, Erratico S, Torrente Y, Meregalli M. Cell based therapy for Duchenne muscular dystrophy. J Cell Physiol 2009;221: 526–534.
99. Kang PB, Lidov HG, White AJ, Mitchell M, Balasubramanian A, Estrella E, et al. Inefficient dystrophin expression after cord blood transplantation in Duchenne muscular dystrophy. Muscle Nerve 2010;41:746–750.
100. Yang XF, Xu YF, Zhang YB, Wang HM, Lu NW, Wu YX, et al. Functional improvement of patients with progressive muscular dystrophy by bone marrow and umbilical cord blood mesenchymal stem cell transplantations [in Chinese]. Zhonghua Yi Xue Za Zhi 2009;89:2552–2556.
101. Zhang C, Chen W, Xiao LL, Tan EX, Luo SK, Zheng D, et al. Allogeneic umbilical cord blood stem cell transplantation in Duchenne muscular dystrophy [in Chinese]. Zhonghua Yi Xue Za Zhi 2005; 85:522–525.
102. Zhang C, Feng HY, Huang SL, Fang JP, Xiao LL, Yao XL, et al. Therapy of Duchenne muscular dystrophy with umbilical cord blood stem cell transplantation [in Chinese]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2005;22:399–405.
103. Huard J, Bouchard JP, Roy R, Malouin F, Dansereau G, Labrecque C, et al. Human myoblast transplantation: preliminary results of 4 cases. Muscle Nerve 1992;15:550–560.
104. Torrente Y, Belicchi M, Marchesi C, Dantona G, Cogiamanian F, Pisati F, et al. Autologous transplantation of muscle-derived CD133+ stem cells in Duchenne muscle patients. Cell Transplant 2007;16:563–577.
105. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–676.
106. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res 2008;103:1204–1219.
107. Kinali M, Arechavala-Gomeza V, Feng L, Cirak S, Hunt D, Adkin C, et al. Local restoration of dystrophin expression with the morpholino oligomer AVI-4658 in Duchenne muscular dystrophy: a single-blind, placebo-controlled, dose-escalation, proof-of-concept study. Lancet Neurol 2009;8:918–928.
108. van Deutekom JC, Janson AA, Ginjaar IB, Frankhuizen WS, Aartsma-Rus A, Bremmer-Bout M, et al. Local dystrophin restoration with antisense oligonucleotide PRO051. N Engl J Med 2007; 357:2677–2686.
109. Alter J, Lou F, Rabinowitz A, Yin H, Rosenfeld J, Wilton SD, et al. Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. Nat Med 2006;12:175–177.
110. Lu QL, Rabinowitz A, Chen YC, Yokota T, Yin H, Alter J, et al. Systemic delivery of antisense oligoribonucleotide restores dystrophin expression in body-wide skeletal muscles. Proc Natl Acad Sci USA 2005;102:198–203.
111. Yokota T, Lu QL, Partridge T, Kobayashi M, Nakamura A, Takeda S, et al. Efficacy of systemic morpholino exon-skipping in Duchenne dystrophy dogs. Ann Neurol 2009;65:667–676.
112. Jearawiriyapaisarn N, Moulton HM, Buckley B, Roberts J, Sazani P, Fucharoen S, et al. Sustained dystrophin expression induced by peptide-conjugated morpholino oligomers in the muscles of *mdx* mice. Mol Ther 2008;16:1624–1629.
113. Wu B, Moulton HM, Iversen PL, Jiang J, Li J, Spurney CF, et al. Effective rescue of dystrophin improves cardiac function in dystrophin-deficient mice by a modified morpholino oligomer. Proc Natl Acad Sci USA 2008;105:14814–14819.
114. Jearawiriyapaisarn N, Moulton HM, Sazani P, Kole R, Willis MS. Long-term improvement in *mdx* cardiomyopathy after therapy with peptide-conjugated morpholino oligomers. Cardiovasc Res 2010;85: 444–453.