Duchenne muscular dystrophy mouse models have a predictable and reproducible time course of cardiomyopathy progression with discrete pathogenic steps, which closely parallel what we know occurs in the cardiomyopathy of patients with Duchenne muscular dystrophy. The slow progression of early pathogenic steps common to many cardiomyopathies may make Duchenne muscular dystrophy models useful for identifying novel treatment targets and testing the therapeutic value of new treatment paradigms for an at-risk patient population far beyond those with muscular dystrophies.

Dilated cardiomyopathy is a leading cause of death in individuals affected by Duchenne muscular dystrophy (DMD), and nearly all DMD patients develop dilated cardiomyopathy by age 18. DMD and the milder Becker muscular dystrophy result from mutations in the X-linked gene encoding dystrophin, a membrane-associated protein that helps protect striated muscle cell membranes from damage. Clinical signs resulting from lower limb muscle weakness lead to DMD diagnosis typically by 5 years of age and loss of ambulation by 12 years of age. Skeletal myopathy in Becker muscular dystrophy has a later age of onset and slower progression than DMD, and dilated cardiomyopathy is the primary cause of mortality. Earlier ventilatory support for affected respiratory muscles and timely management of pulmonary infections have led to increased survival for DMD patients into their mid-twenties. These advances have made increasingly evident the underlying cardiomyopathy that presents as heart failure and often fatal arrhythmias in the later decades of life.

Despite the devastating consequences of DMD cardiomyopathy, its unique features may provide new insights into myocardial disease. First, the consistent lag between evident skeletal myopathy and cardiomyopathy affords early detection and possible treatment of a patient population that is genetically programmed to develop cardiac dysfunction. Second, the myocardial disease exhibits a relatively slow progression, providing the ability to understand each step involved. Third, there are isogenic mouse models of DMD, additional mouse models that have been created to represent a spectrum of dystrophic myocardial disease, and large animal models. Over the past 15 years, these 3 features have allowed the progression of the DMD cardiomyopathy to be functionally dissected in parallel human and mouse studies.

**Steps in Dystrophic Cardiomyopathy**

There are 3 dystrophic mouse models with different severities of myocardial weakness and damage that have been used to dissect out the steps of dystrophic cardiomyopathy. Dystrophin-deficient *mdx* mice are the isogenic model for DMD, but have a late-onset cardiac phenotype that makes studying the pathogenesis and testing treatments a lengthy and expensive process.1 *Mdx* mice also deficient for the dystrophin paralog, utrophin, have the most severe cardiac pathology with reduced myocardial force and extensive cardiomyocyte damage and fibrosis by 10 weeks of age. Working with this model is complicated by their death typically between 10 and 20 week of age resulting from their skeletal muscle pathology.2 *mdx* mice lacking one copy of a functional utrophin gene develop quantitatively more fibrosis in skeletal muscles over time than *mdx* mice and exhibit a faster progression of myocardial weakness and cardiomyocyte damage.3

Using these 3 strains, many studies by our team and others have shown that there are discrete pathogenic characteristics that occur in these models, where initiation of each step reproducibly precedes the initiation of the following step. The first step in the dystrophic cardiomyopathy is myocardial muscle weakness and a blunted β-adrenergic response.4 The next detectable step is cardiomyocyte damage that can be easily observed by the uptake of a vital dye, such as Evan’s Blue, or can be quantified in histological sections by accumulation of serum proteins within cardiomyocytes.5 Cardiomyocyte damage is ongoing with a relatively small percentage of dying cells at any one time point. As this damage progresses, areas of myocyte damage become invaded by fibroblasts producing matrix metalloproteinases, and there is a concomitant reduction in tissue inhibitors of metalloproteinases.6 This process results in replacement of dead cardiomyocytes with fibrotic scars. A remarkable amount of fibrosis accumulates before any reduction in ejection fraction.5,6

A decade of bench-to-bedside-and-back collaboration studying DMD mouse models and patients along with improved imaging techniques has led to substantial advances in understanding DMD cardiomyopathy. Abnormal myocardial strain by cardiac magnetic resonance (CMR) is the first...
detectable sign of cardiac dysfunction in DMD patients. Measuring myocardial strain in dystrophic mice demonstrates a close correlation between in vivo abnormalities in strain and in vitro reductions in myocardial muscle force. In a study of 1768 asymptomatic individuals aged 55 to 75 years who underwent CMR with tagged cine imaging affording strain computation, myocardial circumferential strain (Ecc) was independently and incrementally predictive of symptomatic heart failure over 5 years follow-up in a multiethnic population. Circumferential strain measured by speckle tracking echocardiography in a large cohort of Framingham Offspring Study participants was also found to be predictive of heart failure. Thus, considerable data has accrued supporting myocardial strain abnormalities as an early indicator of developing cardiac dysfunction across a broad range of individuals at risk of cardiomyopathy and heart failure.

The histological damage that is observed as the second step in all dystrophic mouse models is ongoing and seems to involve a relatively slow rate of cardiomyocyte death compared with the massive coordinated cell death present after an ischemic event, for instance. This observation of ongoing damage is supported in DMD patients through episodic detection of elevated troponin-I levels and accrual of myocardial injury by serial late gadolinium enhancement (LGE) imaging. The increases in matrix metalloproteinases-2 and -9 and reductions of tissue inhibitors of metalloproteinases that occur in dystrophic hearts are also conserved across most human heart failure populations and, therefore, have been a target for therapeutic development.

The accumulation of myocardial damage is inevitably replaced by fibrotic scarring. The fibrotic accumulation in the most rapidly progressing DMD mouse model is often in a crescent pattern in the epicardial layer of the left ventricle. However, diffuse fibrosis is typically also present throughout both the left and right ventricles in the mouse models and continues to accumulate throughout both ventricles over the lifespan of the mice. Again, relatively recent advancements in imaging capabilities have led to the ability to detect myocardial damage using CMR with LGE imaging. Notably, all DMD patients show a consistent initial pattern of enhancement in a crescent in the epicardium of the left ventricular free wall. LGE is also predictive of adverse outcomes in other cardiomyopathy patient populations, including patients with nonischemic cardiomyopathy. Importantly, and in parallel with DMD mouse models, this damage in DMD patients is detectable by LGE many years before reduced ejection fraction can be detected by echocardiography. These observations identify a large window for therapeutic interventions for DMD patients and have altered clinical practice in screening for cardiomyopathy and initiating standard-of-care cardioprotective medications, while fueling investigation of therapies that may be beneficial at earlier stages of myocardial disease.

### Bench-to-Bedside–Directed Improvements in Patient Care and Therapeutic Testing

Advances in parallel preclinical and human studies are being incorporated into updated recommendations for monitoring and treatment for DMD patients as well as testing potential therapeutics at earlier stages of disease. A Working Group meeting, sponsored by the National Heart, Lung, and Blood Institute and Parent Project Muscular Dystrophy, was held in summer 2014 to address Contemporary Issues in DMD Cardiomyopathy. This working group updated the DMD Care Considerations to include earlier surveillance using use of CMR to improve the diagnosis and management of DMD cardiomyopathy and for use in monitoring clinical trials in this population. The group also recommended use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers by the age of 10 or earlier.

Using a bench-to-beside approach, our team tested early addition of a mineralocorticoid receptor antagonist, typically used in advanced heart failure, as an anti-fibrotic drug in a DMD mouse model. This study showed that the earliest use of a mineralocorticoid receptor antagonist added to standard-of-care angiotensin-converting enzyme inhibitor resulted in a dramatic reduction in cardiac damage and a dramatic improvement in cardiac strain measurements. We then executed a placebo-controlled double-blind clinical trial, translating this approach to DMD patients with evident myocardial damage by LGE but preserved ejection fraction by cine CMR; this approach attenuated decline of left ventricular circumferential strain in patients with DMD. In addition, there was less reduction in ejection fraction over the course of the study. These data further support the benefit of earlier therapeutic intervention in DMD cardiomyopathy rather than waiting to start treatment after the ejection fraction falls.

### Translation Between DMD Mouse Models and Other Cardiomyopathies

DMD mouse models have a predictable and reproducible time course and relatively slow cardiomyopathy progression that can be separated into discrete steps, which closely parallel data from DMD patients. These characteristics that include reduced myocardial strain, depressed β-adrenergic response, increased matrix metalloproteinase activity, and accumulation of fibrosis are all conserved across a wide variety of cardiomyopathies. Therefore, information about the pathogenesis and treatment in DMD models and patients may be applicable in identifying novel treatment targets and approaches for the broader population of patients with dilated cardiomyopathy.

As an example, we identified a reduction in transcript and resulting protein levels of the claudin-5 gene in the most severe DMD model. This reduction occurs over a 2-week period coincident with the initial onset of cardiomyocyte damage, long before reduced ejection fraction. Exogenous expression of claudin-5 in heart is able to prevent the initial reductions in myocardial force and myocardial damage in this DMD model. We then identified dramatically reduced claudin-5 protein levels in the vast majority of cardiac explant samples from over 75 heart failure patients in comparison with nonfailing human donors. Therefore, molecular...
events occurring early in the progression of dystrophic cardiomyopathy may represent novel treatment targets applicable for a patient population far beyond DMD. Conversely, therapeutics used for other forms of cardiomyopathy or heart failure may be able to target the steps common with DMD cardiomyopathy and improve morbidity. Rapid progression in other mouse models, such as induced pressure overload and coronary artery ligation models, has essential roles for understanding particular disease initiators and may be helpful in understanding symptomatic heart failure, but are less informative when testing therapeutic strategies targeting early pathogenic steps that evolve over years in patients. The reproducibility and slow progression of early pathogenic steps common to many cardiomyopathies support the potential utility of DMD models for identifying novel treatment targets and testing the therapeutic value of new treatment paradigms for a wider patient population.

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References

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Jill A. Rafael-Fortney, Jessica A. Chadwick and Subha V. Raman

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