A workshop was convened on the topic of Cardiomyopathy associated with Muscular Dystrophy in Tucson, Arizona, 28–30 September 2003, sponsored by the Muscular Dystrophy Association. Workshop participants from North America, Europe and Australia set as goals to understand the pathologic basis of cardiomyopathy associated with muscular dystrophy and to review treatment strategies for cardiomyopathy associated with muscular dystrophy. Twenty-five speakers presented data regarding assessment and findings in human subjects with muscular dystrophy, as well as from animal models of cardiomyopathy and muscular dystrophy.

1. Genetics of cardiomyopathy and muscular dystrophy

The muscular dystrophies are genetically heterogeneous with over 25 different genes contributing to the pathogenesis of these inherited disorders [1,2]. Many, but not all, the muscular dystrophy subtypes are associated with cardiac dysfunction that contributes to both the morbidity and mortality of patients with muscular dystrophy [3]. Increased awareness of the cardiomyopathic features in muscular dystrophy will allow timely implementation of existing and novel therapies for cardiomyopathy. This report will briefly outline the presentations and will conclude with recommendations for clinical care and research goals.

Dr Jeffrey Towbin presented an overview of the current genetic status of inherited forms of cardiomyopathy and muscular dystrophy noting the considerable overlap between these two disorders [4]. Many gene mutations lead to both cardiac and skeletal involvement although subclinical involvement may occur. Although remarkable locus and allelic heterogeneity is present in inherited cardiomyopathy and muscular dystrophy, common pathogenetic defects include force transmission and force generation deficits. Mutations in the genes encoding dystrophin and components of the dystrophin glycoprotein complex (DGC) lead to cardiomyopathy that may occur with little to no apparent muscle dysfunction. Duchenne and Becker muscular dystrophies (DMD and BMD) may be accompanied by cardiomyopathy, arrhythmias and congestive heart failure (CHF). Mutations in sarcomere genes can lead to dilated and hypertrophic cardiomyopathies. Mutations in the genes encoding Z band proteins are a newly emerging area to explain inheritance of dilated cardiomyopathies [5–7]. In the last several years, nuclear membrane protein gene defects have emerged as a relatively common cause of cardiomyopathy, cardiac conduction system defects and variable skeletal muscle involvement that can include muscular dystrophy [8]. In addition, dystrophin abnormalities are relatively commonly found in individuals with dilated cardiomyopathy without skeletal myopathy. This occurs due to genetic mutations [9–11] or due to viral infection [12,13] or mechanical stress [14]. The clinical phenotype may be reversible if mechanical unloading therapy, such as with ventricular assist devices, are utilized in a timely fashion [15].

X-linked dilated cardiomyopathy arises from mutations in the dystrophin gene [9,16]. In both DMD and BMD, dystrophin gene mutations produce variable cardiac involvement. The common cardiac findings
include cardiomyopathy that typically develops later than muscle disease [17]. Cardiomyopathy may appear hypertrophic in the early phases and be characterized by systolic and/or diastolic dysfunction. Cardiomyopathy may progress to a dilated phase. Regional wall motion abnormalities may be present and in DMD, may affect the posterobasal segment more than other areas of the heart. Valvular defects may develop from cardiac dilation, and most commonly mitral regurgitation is seen. Arrhythmias may develop along with cardiomyopathy and include both tachyarrhythmias (sinus tachycardia, supraventricular tachycardia (SVT) or ventricular tachycardiac (VT)) and bradyarrhythmias (sinus node dysfunction or atioventricular nodal (AVN) block). Where skeletal muscle weakness is more mild, such as in the case of BMD or female carriers of dystrophin mutations, cardiac transplantation has been successful [18]. The dystrophin-associated proteins, the sarcoglycans, are also implicated in cardiomyopathy and muscular dystrophy with a similar cardiac phenotype to what has been described in DMD and BMD [19].

Mutations in the gene encoding the nuclear membrane protein Lamin A/C have increasingly been described in patients with cardiomyopathy and muscular dystrophy [20]. LMNA gene mutations can produce the phenotype of Emery Dreifuss muscular dystrophy, although the inheritance pattern is autosomal dominant. Rarely, recessive mutations in LMNA can lead to cardiomyopathy and muscular dystrophy [21]. The cardiac findings in LMNA gene mutations may include AVN heart block and/or cardiomyopathy. Howard Worman [8,22] reviewed the range of phenotypes, in addition to cardiomyopathy and muscular dystrophy, associated with LMNA mutations. Lamin A and C are produced from a single gene with alternative splicing at the 3’ end of the gene producing variant carboxyl termini. Some LMNA mutations result in the mislocalization of the nuclear membrane protein emerin. Mutations in the emerin gene lead to X-linked Emery Dreifuss muscular dystrophy. Matt Taylor [23] presented data on families with dilated cardiomyopathy, noting a number of distinct LMNA mutations in these subjects. Therefore, LMNA mutations need not be associated with muscle disease.

Myotonic dystrophy type I (DM1) is not a muscular dystrophy per se, but it is a common human disorder that leads to muscle weakness with cardiac involvement. An expansion of a trinucleotide repeat expansion on chromosome 19 mediates pathology that affects additional tissue types in addition to heart and muscle [24]. The cardiac involvement with DM includes arrhythmias, especially AVN heart block as an early finding, and may include cardiomyopathy. William Groh has been studying the cardiac features in DM patients finding that 65% have an abnormal EKG and 30% have an abnormal Holter monitor recording [25,26]. On echocardiography, findings such as left ventricular hypertrophy, increased left ventricular diameter and decreased left ventricular function are highly prevalent. Sudden death is a significant risk in DM. More recently, a second type of myotonic dystrophy (DM2) was identified related to a genetic defect on chromosome 3. These subjects differ from DM1 in that they have little to no associated cardiac disease.

2. Pathogenesis of cardiomyopathy

Kevin Campbell presented data on the pathogenic mechanisms in DGC-mediated muscular dystrophy noting the tight biochemical association of the DGC components, dystroglycan with sarcoglycan and sarcospan. Dr Campbell highlighted similarities between glycosylation defect disorders including Fukuyama congenital muscular dystrophy, Walker Warburg, muscle eye brain disease and limb girdle muscular dystrophy 2I [27,28]. A common molecular feature of these disorders is defective glycosylation of α-dystroglycan. Sarcoglycan gene defects lead to cardiomyopathy and muscular dystrophy, and mice with null alleles for δ-sarcoglycan and β-sarcoglycan develop these phenotypes [29,30]. Loss of δ- or β-sarcoglycan also results in disruption of the vascular smooth muscle sarcoglycan complex [29,30]. Interestingly, mice lacking α-sarcoglycan have an intact sarcoglycan complex and do not develop cardiomyopathy [31]. Therefore, it was reasoned that the disruption of the vascular smooth muscle complex contributes to the cardiac phenotype in δ- and β-sarcoglycan gene defects. Vascular spasm can be inhibited pharmacologically using verapamil or nicorandil and thereby eliminate cardiomyopathy [32]. However, humans with mutant δ-sarcoglycan induced cardiomyopathy have not been found to have coronary artery abnormalities [33].

Elizabeth McNally presented an alternative view on the origin of vascular defects from DGC mutations. Mice lacking γ-sarcoglycan have an intact vascular smooth muscle complex, yet γ-sarcoglycan mice develop cardiomyopathy and display evidence of vascular defects [34–36]. In this model, defects in the vasculature arise as a consequence of cardiomyocyte damage and may be similarly present in DMD and BMD. The contribution of vascular spasm is significant, since inhibition of vascular spasm was sufficient to partially correct cardiomyopathy progression. Additionally, mislocalization of endothelial nitric oxide synthase is present in sarcoglycan-mediated cardiomyopathy, and this mislocalization leads to functional deficits that can be rescued with inhibitors of nitric oxide synthase [37]. This suggests that the NOS pathway may be selectively overactive.

Jill Rafael-Fortney [38] presented studies on cardiomyopathy in mice lacking both dystrophin and utrophin. These doubly deficient mice were previously reported to have more severe myopathic processes, including the cardiomyopathic process. A series of transgenic mice have been made to restore aspects of the dystrophic protein. For example, restoration with dystrophin Δ17–48 (lacking exons 17 to 48) reduces
the inflammatory component of the dystrophic process. These studies also demonstrate that striated muscle-specific expression is sufficient to rescue the cardiomyopathic process, thereby providing further evidence that cardiomyocyte defects in DMD arise from cardiomyocyte cell-intrinsic defects [39]. Most recently, a comparison of gene expression profiles was undertaken to explore the molecular defects that lead to the more severe cardiomyopathic process in mice lacking both utrophin and dystrophin (unpublished results).

John Quinlan characterized cardiomyopathy present in the dystrophin-deficient mdx mouse. It has been known for some time that cardiomyopathy can develop in the mdx mouse, but the cardiomyopathy appears milder than the human counterpart [40,41]. Like human DMD subjects, cardiomyopathy is not apparent until long after muscle disease is noted. Older mdx mice displayed a slower heart rate and an increase in left ventricular dimensions at 40 weeks of age. Studies performed at earlier timepoints did not demonstrate significant difference from control mice. Ejection fraction was noted to decrease in mdx mice after 42 weeks of age (unpublished results). Fibrosis was seen as a late consequence of the absence of dystrophin in the mdx mouse. Andrew Hoey conducted functional studies of the mdx mouse cardiomyopathy. He noted that mdx mice are hyperresponsive to β blockers and behave as though they have increased sympathetic tone similar to human CHF patients [42]. Using excised left atria from mdx and control hearts, it was noted that higher levels of calcium were required to obtain a similar degree of contraction. Relaxation was delayed in mdx hearts. Certain forms of stress may accelerate the development of dilated cardiomyopathy in mdx mice. Severe kyphosis develops in older animals and may contribute to the development of cardiac and respiratory dysfunctions. Jeff Chamberlain presented data that confirms that the mdx mouse has a reduced lifespan (unpublished results).

Joe Metzger discussed cellular assays to define dysfunction of mdx myocytes. In studies of length–force relationships, mdx cardiomyocytes were found to differ for both passive and active lengthening. In addition, mdx cardiomyocytes did not tolerate lengthening where normal cardiomyocytes did, consistent with enhanced fragility of the dystrophin-deficient myocytes (unpublished results). Robert Bloch from the University of Maryland showed data suggesting that regions of dystrophin may interact with intermediate filaments, and that this interaction may be important for costameric organization in the heart (unpublished results).

Nanette Bishopric reviewed signaling defects associated with cardiomyopathy. The importance of these pathways has been emphasized in heart failure studies since these defects represent important therapeutic targets [43]. The transcriptional activator p300 is essential for normal cardiac transcriptional function. Overexpression of p300 accelerates molecular pathways to hypertrophy while the histone deacetylase (HDAC)/MEF2 pathways act in an opposing manner (unpublished results). Judy Anderson [44,45] presented data indicating the role of neuronal nitric oxide synthase in satellite cell activation. The use of deflazacort is common in DMD patients, and studies in the dystrophin-deficient mdx mouse with deflazacort with L-arginine to increase NO or L-NAME to inhibit NOS showed an increase in utrophin expression.

Viral infections can lead to nongenetic forms of cardiomyopathy. Kirk Knowlton reported that one of the means by which Coxsackie virus mediates cardiomyocyte pathology involves dystrophin [12]. Dystrophin is a target of viral proteases, and also appears to be necessary for viral infection and propagation [46]. Neuronal nitric oxide synthase (nNOS) may interfere with dystrophin mediating infection by inactivating the core of protease 2A (unpublished results).

3. Stem cell therapy

Ken Chien [47] described mice lacking the muscle lim protein (MLP). These mice develop a dilated cardiomyopathy that is dependent on the presence of functional phospholamban [48]. Using viruses that express a pseudo-phosphorylated form of phospholamban, the development of cardiac failure could be blocked in the BIO14.6 hamster model that harbors a δ-sarcoglycan gene deletion [49]. Recent approaches for the treatment of cardiomyopathy have attempted to characterize and exploit stem cells that may be able to develop into cardiomyocytes [50]. The field has been complicated by several different observations. Ideally, a stem cell should be able to be home to the tissue or organ in question and then, once resident at that site, differentiate. Stem cells may be able to divide prior to differentiation, indicating that stem cells may be able to self renew. Once stem cells differentiate into skeletal muscle cells, for example, they lose their ability to proliferate and renew. While stem cells have been shown to contribute to skeletal muscle regeneration, it is now clear that caution in interpreting these studies is needed. Some stem cells acquire the appearance of differentiation when, in fact, these cells simply fused to already existing mature cells. In skeletal muscle, this may not be problematic since cell fusion is the means by which myoblasts fuse to form mature myotubes. Therefore, fusion of stem cells to myotubes may still improve muscle function.

Cardiomyocytes, on the other hand, are not syncytial. Therefore, it is not clear whether stem cell fusion would be expected to benefit a mature cardiomyocyte [51–53]. Fusion is also complicated since a stem cell may fuse to an existing mature cell with or without concomitant nuclear fusion. Cytoplasmic fusion without nuclear fusion leaves a cell with two nuclei of disparate maturation status. In the event that cytoplasmic fusion is followed by nuclear fusion, the resulting tetraploid nucleus would likely have aberrant
function with regard to gene expression. These issues mandate the use of appropriate cellular markers when performing stem cell transplant experiments to fully appreciate how effective stem cell therapy may be in the treatment of both cardiomyopathy and muscular dystrophy. Along those lines, Dr. Chien is using markers of early cardiomyocyte development to study cardiomyocyte development in vitro. These studies may be very useful for future cardiac stem cell biology.

Dan Garry discussed molecular characteristics of stem cells [54]. From bone marrow and other tissues, a population of stem cells was previously identified based on the ability to efflux Hoechst dye [55,56]. These cells, referred to as side population or ‘SP’ cells can also be identified from cardiac muscle. Gene expression profiling of SP cells suggests that SP cells differ considerably from tissue to tissue. Dr. Garry’s group now used a transgenic strategy where the NKX2.5 promoter, a promoter expressed early in cardiomyocyte specification, was used to drive expression of yellow fluorescent protein. Isolation of fluorescent cells from the early developing heart identified a population of progenitor cells that can be characterized by upregulation of the gene ATP binding cassette subfamily G member 2 (ABCG2) (unpublished results). ABCG2 is involved in drug resistance and may directly mediate the dye efflux used to identify these cells [57].

4. Viral strategies for correcting cardiomyopathy in muscular dystrophy

Viral gene therapy has been explored for muscular dystrophy, and current emphasis centers on adeno-associated virus (AAV). The safety profile favors these viruses, and studies in mice have shown a capacity for long-term stable expression. Jeff Chamberlain generated high capacity adenoviruses expressing dystrophin and demonstrated expression as long as 6 months of age in the mdx mouse [58–60]. Infection with high capacity adenovirus expressing utrophin revealed longer term expression suggesting immune response to dystrophin may account for the comparative decline in dystrophin expression. With this approach, it was shown that the dystrophin rod region is required to correct the dystrophic process. The same adeno-viral constructs may show differential effects on different muscle groups.

Dongsheng Duan has been evaluating AAV gene therapy for the treatment of cardiomyopathy in DMD and BMD. As AAV is limited in its capacity for large fragments of DNA, micro and minidystrophin constructs have been used. AAV designed to express microdystrophin under a broadly expressed promoter was introduced into newborn mdx hearts. Using this approach, viral transduction in the epicardial layers was seen; AAV serotype 5 was found to be more effective [61]. Future functional studies are planned.

Hansell Stedman and Charles Bridges are working to establish improved delivery methods for both skeletal and cardiac muscle viral gene therapy. Widespread viral transduction has proved difficult to obtain with both intra-arterial and intramuscular injections. By modifying existing intravenous approaches, widespread skeletal muscle transduction was obtained in a large animal model. A further modification of this approach was used to transduce hearts, again with good results [62]. Barry Byrne [63] discussed the timeframe required to move viral gene therapy to human trials, and the importance of good animal models in which to conduct essential preclinical trials.

5. Clinical recommendations

The European Neuromuscular Centre convened a meeting in 2002 on Cardiomyopathy and Muscular Dystrophy [64]. Kate Bushby presented the findings and recommendations from that gathering. At the 2002 meeting and the present meeting, it was agreed that clinical studies were not available to provide specific recommendations regarding the management of cardiomyopathy in muscular dystrophy. The rare nature of these disorders leads to difficulty in assembling sufficient numbers of subjects for controlled clinical trials. As such, multicenter-based trials are needed, and multicenter trials are just beginning. The complexity in conducting these trials also includes the determination of adequate endpoints and uniformity in performance and interpretation of noninvasive studies. Carolyn Spencer reviewed the use of echocardiography as a tool to predict outcome in subjects with cardiomyopathy. In some studies, left ventricular function has been used as an endpoint for studies of heart failure, and it may be useful for studies of the heart failure that accompanies muscle disease.

Paula Clemons is working with Cooperative International Neuromuscular Research Group (CINRG) to begin a study of the efficacy of Co enzyme Q10 and prednisone in children with DMD [65]. An arm of this study will evaluate the effect of these medications on cardiac function including wall stress, shortening fraction, and tissue Doppler imaging. Valerie Cwik discussed the ongoing MD STARNet study sponsored by the Centers for Disease Control. Its goals are to determine whether there has been a change in the prevalence of DMD and to determine what treatment is being offered to children with DMD.

Until controlled clinical trials are available for cardiomyopathy with muscular dystrophy, recommendations for clinical care should rely on controlled clinical trials of heart failure and cardiomyopathy. The mainstay of pharmacologic treatment for left ventricular dysfunction for adults with cardiomyopathy and CHF relies on (1) afterload reduction with ACE inhibitors as a first line
option and angiotensin receptor blockade if ACE inhibitors cannot be used, (2) beta adrenergic receptor blockade, (3) spironolactone, (4) diuretics as needed to manage fluid overload, and (5) aggressive surveillance for cardiac arrhythmias. The role of additional pharmacologic agents such as digoxin is not clear. Arrhythmias, if detected or suspected, can be treated by device implantation and/or pharmacologic therapy with anti-arrhythmic agents. Surveillance should include regular EKGs, Holter monitoring and/or event monitoring. Syncpe, if noted, may warrant treatment for presumed arrhythmias. Similar therapies in children, with the use of ACE inhibitors and β-blockers with or without diuretics and digoxin, are first line approaches.

The specific recommendations may be modified with regard to the precise gene defect. In DMD, heart involvement generally occurs later than skeletal muscle involvement and may not be present until the late second decade. It is not known at this time whether early treatment, before the visible onset of left ventricular dysfunction may slow the course of cardiomyopathy. Future clinical trials should be designed to determine at what age and at what stage therapy to prevent cardiomyopathy should be initiated. As these problems can become evident in the early second decade, monitoring should begin at that time. Cardiologists, whether specialized in the care of adult or pediatric patients, should be experienced in caring for subjects with muscular dystrophy. The Muscular Dystrophy Association may wish to identify a referral base of cardiologists who work with the MDA clinics throughout the country.

In BMD, cardiac involvement may occur later but may eventually become a prominent feature. Cardiac transplantation can be offered if pharmacologic therapy fails. Monitoring should begin in the late second decade for BMD. Finally, female carriers of dystrophin mutations should be evaluated beginning in their late 3rd to 4th decade since cardiomyopathy may develop in these subjects.

LMNA mutations increase the risk of cardiomyopathy, CHF and sudden death. In these subjects, cardiac involvement may be present with little or no skeletal muscle disease. As such, a knowledgeable cardiologist should examine these patients routinely to determine whether a pacemaker or automatic defibrillator is required. Genetic testing and counseling of family members at risk should be offered. Presently, children carrying LMNA gene defects may be at some risk beginning in the late second decade. Additional studies are required to determine at which point therapy should begin for these subjects.

Myotonic dystrophy type 1 subjects require surveillance for arrhythmias and echocardiography for ventricular dysfunction. Prolonged PR prolongation is an American Heart Association class II recommendation for pacemaker implantation. For both myotonic dystrophy patients, like LMNA subjects, sudden death may still occur despite pacemaker implantation. This observation most likely indicates the presence of ventricular tachyarrhythmias.

For more rare forms of muscular dystrophy, including sarcoglycan gene mutations and emerin gene mutations, the recommendations are similar to DMD and LMNA gene mutations, respectively. For many muscular dystrophy patients for whom their gene mutation is unknown, routine evaluation by a cardiologist with regular echocardiography, EKG and Holter monitoring is warranted to identify and institute early treatment for accompanying cardiovascular disease. In addition, as many muscular dystrophy subjects are living longer, routine assessment for the more common cardiovascular diseases, such as coronary artery disease, should not be forgotten.

Genetic testing should be sought for all muscular dystrophy patients since it has direct bearing on the risk of cardiovascular involvement. Those subjects with dysferlin, calpain and caveolin gene mutations are thought to have reduced risk of cardiovascular involvement. Those subjects with the trinucleotide expansion at the DM1 locus or with gene mutations in dystrophin, lamin A/C, sarcoglycan or emerin are at greater risk. Genetic testing is commercially available for a small number of these disorders, and false negatives remain problematic. Therefore, priority should be to provide a genetic diagnosis for all subjects and relatives at risk with muscular dystrophy.

6. Summary

The meeting was felt to be a highly productive gathering of scientists and clinicians and served to increase the awareness and need to study and treat the cardiomyopathy associated with muscular dystrophy. Animal models are now available for many of these disorders and provide an important forum for testing new and existing therapies for treating cardiomyopathy with muscular dystrophy. It was suggested that additional meeting be held in the future to mark progress in these goals.

7. Workshop participants

Elizabeth McNally, Co-chair, Chicago, IL
Jeffrey A. Towbin, Co-chair, Houston, TX
Ronald Allen, Tucson, AZ
Judy Anderson, Winnipeg, Manitoba, Canada
Robert J. Bloch, Baltimore, MD
Charles R. Bridges, Philadelphia, PA
Katherine Bushby, Newcastle upon Tyne, UK
Barry J. Byrne, Gainesville, FL
Kevin P. Campbell, Iowa City, IA
Charles Canter, St. Louis, MO
Jeffrey S. Chamberlain, Seattle, WA
Kenneth R. Chien, La Jolla, CA
Paula R. Clemens, Pittsburgh, PA
Ronald D. Cohn, Baltimore, MD
David M. Connuck, Rochester, NY
Linda H. Cripe, Cincinnati, OH
Chris Cunniff, MD, Tucson, AZ
Valerie A. Cwik, MD, Tucson, AZ
Dongsheng Duan, Columbia, MO
R. Erik Edens, Iowa City, IA
Gordon Ewy, Tucson, AZ
Kevin M. Flanigan, Salt Lake City, UT
Daniel J. Garry, Dallas, TX
Kathryn A. Glatter, Sacramento, CA
William J. Groh, Indianapolis, IN
Michelle Henricks, Los Angeles, CA
Andrew Hoey, Queensland, Australia
R. Rodney Howell, Miami, FL
Kirk U. Knowlton, La Jolla, CA
William R. Lewis, MD, Sacramento, CA
Heather MacLeod, Chicago, IL
Larry Markham, Cincinnati, OH
Katherine D. Mathews, Iowa City, IA
F. John Meaney, Tucson, AZ
Joseph M. Metzger, Ann Arbor, MI
John Quinlan, MD, Cincinnati, OH
Kumaraswamy Sivakumar, Phoenix, AZ
Carolyn Taylor Spencer, Gainesville, FL
Hansell H. Stedman, Philadelphia, PA
Dietrich Stephan, Tempe, AZ
Lawrence Stern, Tucson, AZ
Matthew Taylor, Aurora, CO
James Tidball, Los Angeles, CA
Matteo Vatta, Houston, TX
Robert Weiss, Iowa City, IA
Howard J. Worman, New York, NY

References


