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Left Ventricular Dysfunction in Duchenne Muscular Dystrophy and Genotype

Mahi L. Ashwath, MD^a, Irwin B. Jacobs, MD^a, Carol A. Crowe, MD^a, Ravi C. Ashwath, MD^b, Dennis M. Super, MD, MPH^a, and Robert C. Bahler, MD^a

^aMetroHealth Medical Center, Case Western Reserve University School of Medicine, Cleveland, Ohio

^bRainbow Babies and Childrens Hospital, Case Western Reserve University School of Medicine, Cleveland, Ohio

Abstract

Prognosis in patients with Duchenne muscular dystrophy (DMD) is guarded and most deaths are due to cardiac or respiratory causes. It is unclear if some *DMD* gene mutations might be predictive of either mild or severe cardiac dysfunction. We studied 75 patients with DMD followed at our institution. Cardiac function, as assessed by yearly echocardiography, showed marked variability in left ventricular (LV) function. Some patients in their 3rd decade had no or minimal dysfunction whereas others in their 2nd decade had very severe dysfunction. Therefore, 4 Severity Groups were defined ranging from no/mild LV dysfunction to severe LV dysfunction using patient age at first abnormal echocardiogram and degree of LV dysfunction. Genetic data were collected for all patients. The majority of patients had mutations between exon 1 – 20 and exon 41 – 55. The distribution of the 4 Severity Groups of LV dysfunction did not significantly differ among these two mutation groups. An analysis based on the number of exons involved (< 5 exons versus ≥ 5 exons) also found no significant difference in cardiac severity. When patients having identical mutations were compared as to their cardiac course, concordance was often not evident. Steroid therapy had no apparent protection for the development of cardiomyopathy. In conclusion, 75 patients with DMD showed marked variability in the severity of LV dysfunction. Neither age of onset nor severity of cardiomyopathy correlated with any of the mutation groups.

Keywords

Duchenne muscular dystrophy; cardiomyopathy; echocardiography; dystrophin

Corresponding Author: Robert C. Bahler, Heart and Vascular Center, MetroHealth Medical Center, 2500 MetroHealth Drive, Cleveland, OH 44109, Fax Number: 216 778-3927, Phone Number: 216 778-2431, rbahler@metrohealth.org.

Disclosures

None

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Introduction

Duchenne muscular dystrophy (DMD) results in skeletal and cardiac muscle dysfunction and is caused by mutations in the *DMD* gene located at Xp21.1.¹ Most mutations are deletions^{2–4} and result in the absence or minimal amounts of dystrophin, a subsarcolemmal protein product first identified in 1987.⁵ As a consequence, cardiac dysfunction and respiratory muscle weakness develop and often result in premature death in the second or third decade of life.⁶ Most patients develop myocardial disease characterized by early and extensive fibrosis in the basal inferolateral wall of the left ventricle (LV) and later, the lateral free wall.^{7–10} Although cardiomyopathy develops in at least 90% of patients, the age when this becomes clinically evident is variable.^{11–13} Several prior reports suggested that some *DMD* mutations might be predictive of more severe cardiac involvement¹³ and some mutations might be cardio protective.¹⁴ However, no study has specifically focused on characterizing the marked variability in onset and severity of LV dysfunction, and whether this variability is predictable by genotype. The clinical implications of such correlations would be significant with the potential of a specific genotype predicting patients at either high or low risk of early LV dysfunction. Conversely, absence of any correlation would not allow predictions of risk.

Methods

Patients (n = 124) with a dystrophinopathy who were followed in the MetroHealth Medical Center Muscular Dystrophy Association Clinic over the years 1999–2011 were eligible for inclusion in the study. Patients with DMD and Becker muscular dystrophy (BMD) have similar *DMD* mutations and patients with BMD often develop severe cardiac dysfunction. Nevertheless, those with BMD were excluded from the current study leaving 104 patients having DMD, none of whom were walking at 12 years of age. Additional exclusion criteria were age less than 10 years at their last visit or inadequate echocardiographic data. The remaining 75 patients with DMD are the focus of this study. Demographic and clinical data including age, race and the genetic mutation were collected on all the patients. The hospital Institutional Review Board approved the study.

Cardiac function was assessed by echocardiography. One co-author (MLA), blinded to the specific genotype, reevaluated and measured all echocardiograms to obviate interobserver variability. Standard cardiac imaging was performed using either Philips Sonos 500 or Philips iE33 (Andover, Mass) imaging systems and repeated at approximately yearly intervals. Images were stored in a digital format. The LV internal dimension at end-diastole (LVIDd) and the LV internal diameter at end-systole (LVIDs) were measured from the 2-dimensional parasternal long axis view. End-diastole was defined as the largest diameter prior to the onset of shortening so as to avoid the diastolic compression of the posterolateral wall from gastric pressure. Three cardiac cycles were measured for each study and the mean values were used to calculate the left ventricular shortening fraction (SF) expressed as a percent: $\{[(LVIDd - LVIDs)/LVIDd] \times 100\}$.

Although cardiomyopathy may be evident by measurements of circumferential strain, tissue backscatter and myocardial tissue velocities prior to reductions in ejection fraction or

SF,^{15–17} we considered left ventricular (LV) dysfunction and cardiomyopathy to be present only if the SF was <29% and focal hypokinesis was present. Shortening fraction rather than ejection fraction was chosen for the serial observations because: 1) ejection fraction is best calculated from apical images but the quality of apical images in patients with DMD markedly deteriorates with increasing age due to loss of intercostal windows; 2) SF incorporates the function of the basilar inferolateral wall which is the earliest and dominant region of LV dysfunction in almost all patients, thus, the SF may be reduced when the LV ejection fraction is normal..

When SF was normal throughout the period of observation, the patient's age at the last normal SF was noted; when the SF was abnormal, the patient's age at the first abnormal SF was noted. In order to integrate age with the variability of SF, a rating scale to reflect the severity of cardiac dysfunction was developed based on both the age at onset of LV dysfunction and the severity of dysfunction as determined by the SF at the patient's first abnormal echocardiogram. Left ventricular function was defined as follows; normal = SF 29% and no wall motion abnormalities, minor LV dysfunction = SF 24–28% with wall motion abnormalities and major dysfunction = SF < 24%. This scale resulted in the following 4 *a priori* Severity Groups (Table 1). Group 1 was indicative of no or minimal cardiac involvement during the 2nd and 3rd decades. Group 4 was indicative of severe cardiac involvement as reflected by major cardiac dysfunction at an early age. Five patients < 15 yrs old and with normal LV function were not included in the analyses of the Severity Groups nor in the prevalence of cardiomyopathy since their subsequent cardiac future could not be predicted. However, these patients were included in the survival analyses since they would be treated as a censored case for the time period beyond their last echo.

The *DMD* gene mutation analyses were performed by a variety of techniques, depending on the methods that were clinically available in the year in which they presented for genetic evaluation or re evaluation. Beginning in the early 1990s, the patients were tested for duplications and deletions via PCR and Southern Blots using standard techniques. When the mutation was not identified, standard *DMD* gene sequencing was performed when the technology became clinically available. Those without a definitive result had blood analyzed using a comparative genomic hybridization array that was custom designed for analyzing the *DMD* gene. This technique became clinically available in the mid 2000's. If no mutation was found, the patient underwent a muscle biopsy. If dystrophin was not present, they were presumed to have DMD, but were not included in the study, as their mutation remained unknown. Currently, patients are tested first with the dystrophin microarray and then the gene is sequenced. Tests in the 75 patients were PCR/Southern Blood [52 (69%)], Sequencing [7 (9%)], Dystrophin Array [6 (8%)] and 10 (13%) siblings were not tested.

Patients were divided into 4 genetic groups based on the first exon involved in the mutation. Group A with mutations starting from exon 1 – 20, Group B with mutations starting from exon 21 – 40, Group C with mutations starting from exon 41 – 55 and Group D with mutations starting at any level greater than exon 55. In a separate analysis based on the number of exons involved, patients were classified as having fewer than five exons (<5E) or equal to or greater than five exons (≥ 5E) in their mutation.

The data are described using percentages (nominal data), medians with interquartile ranges (IQR, ordinal or nonparametric interval data) and means with standard deviations (parametric interval data). Parametric interval data are defined as having both skewness and kurtosis between -3.00 and $+3.00$. Differences between groups for unpaired data were assessed by the following statistical tests: Chi square or Fishers Exact Test (nominal data); Mann Whitney U test (ordinal or nonparametric interval data); and either the pooled or separate variance T-test depending on the F value of the variances (parametric, interval data). For paired data the Wilcoxon Sign Rank Test (ordinal data) and the paired T-test (parametric interval data) were used. Kaplan Meier analyses were used to compare the survival curves (time of onset of cardiomyopathy) regarding both steroid usage and the genetic data. The log rank test was used to compare survival curves when the assumption of proportional hazards was met. If that assumption was not met (i.e., curves intersect), then the Breslow test was used. The survival curves are summarized as the median time of onset of cardiomyopathy with the corresponding 95% confidence interval. Linear regression analysis was used for generating the Pearson R values. The residuals were examined to determine if all the assumptions of regression modeling were met (if not, then appropriate transformations were made). The data were analyzed with IBM SPSS Statistics version 20 (IBM Inc., Armonk, New York). Statistical significance was defined a priori as a “p” value < 0.05 (two-tail).

Results

The clinical, genetic and cardiac features of the 75 male patients are shown in Table 2. Most patients were Caucasian. Deletions were the most common type of mutation. The largest genetic group was C with the mutation starting from exon 41 – 55, and the majority of patients had fewer than five exons involved.

Cardiomyopathy was evident in 43 (57.3%). The prevalence of cardiomyopathy increased with age with the median age for the onset of cardiomyopathy being 21.0 years (95% CI: 18.2 – 23.8 years) (Figure 1). There was marked variability in the SF at the time of diagnosis of the cardiomyopathy ranging from 11% to 28% (21.2 ± 3.9). There was no correlation of age at onset and the SF at the time of diagnosis of cardiomyopathy ($n = 43$; $r = -0.08$, $p = 0.61$) nor were age and SF related amongst those without evidence of cardiomyopathy (SF 29) ($r = 0.19$, $p = 0.30$) (Figure 2). These observations were not affected by cardiac therapies since the Muscular Dystrophy Association Clinic initiated cardiac medications only after there was echocardiographic evidence of LV dysfunction.

The mutations in the *DMD* gene for all DMD patients, stratified by the cardiac disease Severity Groups, are presented in Figure 3. The distribution of mutations appeared similar for the 4 Severity Groups. The majority of patients had mutations between either exon 1 – 20 ($n=21$) or exon 41 – 55 ($n=42$) and the distribution of the 4 Severity Groups of LV dysfunction did not significantly differ among these two mutation groups [3 (1.0 to 3.5) versus 2.5 (1.0 to 3.0); median (interquartile range), $p=0.76$; Table 3]. An analysis based on the number of exons involved [< 5 exons ($n=43$) versus ≥ 5 exons ($n=27$)] also found no significant difference in cardiac severity [1 (1.0 to 3.0) versus 3 (1.0 to 3.0); median (interquartile range), $p=0.224$]. Specific statistical comparisons for the different genetic

groups confirmed no difference in prevalence of cardiomyopathy, severity of the cardiomyopathy or the age of onset of cardiomyopathy in relationship to the location of the first exon mutation (< 40 , > 40), exon grouping (0–20, 41–55) or number of exons involved (< 5 , > 5) ($p > 0.05$; Table 3). There was no association between the Severity Groups and whether the exon alteration was a deletion or duplication. The exon deletions that included 48–49 and 51–52 that previously were suggested to be either associated with more severe disease¹³ or to be cardioprotective¹⁴ were randomly distributed among the 4 Severity Groups (Figure 3).

The cardiac status was compared among patients having the same mutations; i.e. the specific locations of the deleted exon(s) were alike. Some of these patients were siblings or cousins while others were unrelated. The study included 3 sets of 3 brothers, 4 sets of 2 brothers; two of the sets of 3 brothers were cousins to each other. Twelve different mutations were present in more than 1 patient. The specific mutations, the number of patients affected and the Severity Group are listed (Table 4). Of the 2 mutation patterns found in more than 3 patients, neither deletion of exon 44 nor deletion of exon 51 was associated with a specific cardiac Severity Group (either mild or severe left ventricular dysfunction). Even though these observations may be of interest, additional subjects are needed to show statistical significance of these observations.

Amongst our DMD cohort, 11 of 75 (14.7%) patients received at least 1 year of steroid therapy which was initiated prior to 10 years of age. All 11 had a normal echocardiogram prior to steroid therapy. The prevalence of cardiomyopathy was similar in those who received steroids in contrast to those who did not (6/9, 66.7% versus 37/61 60.7%, $p = 0.73$ respectively). The Severity Group score was also similar in those who received steroids and those who did not [3.0 (1.0, 4.0) versus 2.0 (1.0, 3.0); $p = 0.25$, respectively]. In comparing the survival curves for onset of cardiomyopathy, the steroid group developed their cardiomyopathy at a younger age [mean 18.8 (14.8, 22.8) versus 24.9 (22.3, 27.6, $p = 0.008$)]. The age of the last normal echocardiogram was also younger in the steroid group (14.3 ± 5.0 versus 19.1 ± 5.9 years, $p = 0.017$).

Discussion

Our study is the largest study to date from a single institution to characterize the marked variability of cardiac dysfunction in patients with DMD. Since no prior reports systematically assessed the relative severity of the cardiomyopathy, we introduced a scale to reflect this variable severity; this classification encompassed both the age of onset and a quantitative assessment of the degree of LV dysfunction. We observed that some young patients had severe LV dysfunction and some older patients had apparently normal LV function. Importantly, this variability in severity of LV dysfunction appeared unrelated to the location of the genetic mutation within the *DMD* gene. The severity of cardiomyopathy, in contrast to just evidence of cardiomyopathy, is relevant to both patient care and clinical outcome. Cardiac dysfunction can cause disability and death in the 2nd decade or be delayed well into the 3rd or 4th decade. However, contemporary genetic testing did not provide predictive information as to the onset or severity of LV dysfunction.

Prior studies of the dystrophinopathies tended to focus on the presence or absence of cardiac disease rather than the age of onset and severity of LV dysfunction.^{13, 18, 19} Cardiac dysfunction was usually determined by echocardiography but sometimes the electrocardiogram and Holter monitoring were used to determine the presence of cardiomyopathy. However, specific electrocardiographic abnormalities that are common in DMD do not correlate with the presence of cardiomyopathy as assessed by echocardiography.²⁰

Our results are not in agreement with a prior study of 47 patients with echocardiographic and genetic data, which suggested that some genotypes (mutations of exons 51–52), were cardio protective¹⁴ and others (that included exons 48–49) were associated with earlier onset of cardiomyopathy.¹³ Only 3 of our patients with deletions of exons 51 and 52 were free of cardiomyopathy (severity Group 1) and 5 had advanced disease (Groups 3 and 4) as shown in Figure 3. Also, many of our patients in severity group 1 had mutations in the exons that had been suggested to be associated with early cardiomyopathy.¹³ Differences between our study and prior studies may relate to our larger cohort and a substantially longer follow up

Prior studies have noted phenotype variability within families of brothers with DMD. A report of intrafamilial DMD noted a variable clinical course.²¹ Our data in patients with the same mutations including brothers and cousins with an identical mutation emphasizes the limited concordance between a specific mutation and the severity of the cardiomyopathy. Also, the severity of respiratory dysfunction does not correlate with the severity of cardiac dysfunction.²² Other investigators have shown heterogeneity in the clinical presentation of patients with DMD. No significant genotype/phenotype correlations were found apart from cognitive abnormalities that were more common with mutations above exon 63.²³ Thus, the current use of genotyping in patients with DMD should be viewed primarily as a means to determine the presence or absence of a dystrophinopathy.

The reasons for a lack of concordance in the cardiac course among patients with the same genetic mutations remain uncertain. A possible explanation is in the similarities or differences in the remainder of the genetic environment inherited in each patient. One example of a variable milieu effecting the progression of DMD relates to the activity of the gene *SPP1* which codes for the protein osteopontin.²⁴ Individuals with particular polymorphisms of rs28357094 in the promotor region of this gene have differing disease progression. Those with the rare G allele (either GT or GG) had more rapid disease progression. It was concluded that the GT and the GG variations of this polymorphism decreased *SPP1* mRNA production and this in someway plays a part in influencing the disease course in DMD. In the mouse model *SPP1* modulates muscle inflammation and regeneration.²⁴ Stratifying research subjects with DMD as to their rs28357094 status may help clarify unexplained results in natural history studies and clinical trials.

Our observations emphasize the uncertainty regarding conclusions as to therapeutic efficacy of interventions to improve cardiac function or delay the onset of dysfunction when the studies involve only a modest number of patients with DMD.^{25–26} For example, if the majority of patients receiving active therapy were in severity group 1 and the majority receiving placebo were in severity group 4, it would appear as if a major benefit occurred

with active therapy. This concern includes the role of steroid therapy since our patients who received steroid therapy had a severity of cardiac dysfunction similar to those not treated, yet the onset of cardiomyopathy was at an earlier age. This observation is in contrast to prior reports.^{27,28,29} Future therapeutic studies must assure participation of a very sizeable population to avoid this pitfall.

Limitations to our study include a possible patient selection bias as a single center study. However, a single center study has the advantages of consistent and longitudinal assessment of LV function. Prospective observational data from a very large cohort might be able to show some statistically significant associations between each specific genotype and severity of cardiomyopathy but our data indicate that it is unlikely that there would be strong associations. It should be emphasized that echocardiographic examination of these patients in wheelchairs, most of whom had spinal fusion and many of whom have become obese, is challenging and could lead to errors in the measurement of SF.

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Figure 1. Cumulative survival without echocardiographic evidence of cardiomyopathy as related to age. Note that not all patients with DMD have echocardiographic evidence of cardiomyopathy at age 25 years.

Figure 2.

Shortening fraction (SF) as related to patient age. The patient's age at their last normal echocardiogram is displayed for those patients with a persistently normal SF (SF $\geq$ 29). When patients had an abnormal SF, the age at the first abnormal SF is displayed. The distribution of patients with either normal SF or abnormal SF is unrelated to age. The variability of SF is marked.

Figure 3.

Map of the mutations within each of the four Severity Groups. The exon numbers are shown at top. The length of each line corresponds to the number of involved exons. Note that the distributions of the exon mutations are similar for the 4 severity groups. Three patients had alterations above exon 55; 1 patient in severity class 1 (nonsense at exon 64), 1 patient in Severity Group 2 with a splice site mutation at exon 70 and 1 patient in Severity Group 3 with a deletion at exon 65.

Table 1

Severity Groups as Related to Patient Age and Degree of Left Ventricular Dysfunction

<u>Severity Group</u>	<u>Age</u>	<u>Degree of Left Ventricular Dysfunction</u>
1	15	None
1	>19	Minor
2	<15	None
2	15–19	Minor
3	<15	Minor
3	>19	Major
4	19	Major

Table 2**Patient Characteristics (n = 75)**

Race	
European American	70 (93%)
African American	3 (4%)
Hispanic	2 (3%)
Mutation type	
Deletion	54 (72%)
Duplication	14 (19%)
Nonsense	4 (5%)
Frame shift	2 (3%)
Splice site	1 (1%)
Medication usage	
ACE-inhibitor*	45 (60%)
Angiotensin receptor blocker*	3 (4%)
Beta-blocker*	26 (35%)
Digoxin*	18 (24%)
Steroid	11 (15%)
Location of mutations	
Group A (exon 1– 20)	22 (29%)
Group B (exon 21 – 40)	3 (4%)
Group C (exon 41–55)	46 (61%)
Group D (exon > 55)	4 (5%)
Number of mutations	
Group L5E (< 5 exons)	47 (63%)
Group G5E (≥ 5 exons)	28 (37%)
Severity of Cardiomyopathy	
Severity Group 1	31 (41%)
Severity Group 2	10 (13%)
Severity Group 3	21 (28%)
Severity Group 4	13 (17%)

* Initiated after evidence of LV dysfunction

Table 3

Association of Genetic Mutation and Cardiomyopathy

Exon Location	40 (n = 24)	>40 * (n = 46)
Cardiomyopathy (%)	71%	57%
Severity Group (median, IQR)	2.5 (1.0, 3.8)	2 (1, 3)
Median Time (years) [†]	25.6 (21.2, 30.0)	21.6 (19.7, 23.5)
Exon Grouping	0–20 (n = 21)	41–55 * (n = 42)
Cardiomyopathy (%)	71%	60%
Severity Group (median, IQR)	3 (1.0, 3.5)	2.5 (1, 3)
Mean time (years) [†]	21.0 (12.9, 29.1)	21.0 (18.5, 23.5)
Exons Involved	< 5 exons (n = 43)	5 exons * (n = 27)
Cardiomyopathy	54%	74%
Severity Group (median, IQR)	1 (1, 3)	3 (1, 3)
Median Time (years) [†]	23.0 (19.1, 26.9)	21 (19.8, 22.2)

* All p values in this section are > 0.05

[†] Median or mean age for development of cardiomyopathy (95% CI). Five additional patients who were < 15 years of age and with normal cardiac function were included in the survival analyses.

Table 4

Severity of Cardiomyopathy with Mutations Involving the Same Exon(s)

Genetic mutation	Number	Severity Group
Duplication Exon 2	3	1, 1, 2
Duplication Exons 2, 3*	2	3, 4
Deletion Exons 3 – 7	3	1, 1, 3
Deletion Exon 44*	9	1, 1, 1, 1, 2, 3, 3, 3, 4
Deletion Exon 45	2	2, 2
Deletion Exons 45 – 48	3	1, 2, 2
Deletion Exons 45 – 50*	3	3, 4, 4
Deletion Exons 46 – 49	3	1, 1, 2
Deletion Exon 51*	5	1, 2, 3, 3, 4
Nonsense Exon 52*	2	3, 3
Deletion Exons 53 – 59	2	1, 3

* Some data previously reported in 15 siblings and cousins.²²