Cardiac Dysfunction in Duchenne Muscular Dystrophy Is Less Frequent in Patients With Mutations in the Dystrophin Dp116 Coding Region Than in Other Regions

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BACKGROUND: Duchenne muscular dystrophy (DMD), the most common inherited muscular disease in childhood, is caused by dystrophin deficiency because of mutations in the *DMD* gene. Although DMD is characterized by fatal progressive muscle wasting, cardiomyopathy is the most important nonmuscle symptom threatening the life of patients with DMD. The relationship between cardiac involvement and dystrophin isoforms has not been analyzed.

METHODS AND RESULTS: The results of 1109 echocardiograms obtained from 181 Japanese DMD patients with confirmed mutations in the *DMD* gene were retrospectively analyzed. Patients showed an age-related decline in left ventricular ejection fraction. Patients were divided by patterns of dystrophin isoform deficiency into 5 groups. The cardiac dysfunction-free survival was significantly higher in the group with mutations in the Dp116 coding region than the others, whereas no significant differences in the other 3 groups. At age 25 years, the cardiac dysfunction-free rate was 0.6 in the Dp116 group, but only 0.1 in others. PCR amplification of Dp116 transcript in human cardiac muscle indicated promoter activation.

CONCLUSIONS: Left ventricular ejection fraction in DMD declined stepwise with age. Cardiac dysfunction was less frequent in Dp116-deficient than other patients with DMD. Dp116 transcript was identified in human cardiac muscle for the first time. These results indicate that Dp116 is associated with cardiac involvement in DMD.

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Clinical Perspective

Duchenne muscular dystrophy (DMD) is the most common inherited muscular disease in childhood and is characterized by fatal progressive muscle wasting. The main cause of early death of DMD is cardiomyopathy caused by dystrophin Dp427 deficiency. Currently, management of cardiomyopathy is the most important issue in patients with DMD. In this study, the results of 1109 echocardiograms obtained from 181 Japanese DMD patients with confirmed mutations in the *DMD* gene were retrospectively analyzed. Patients were divided by patterns of dystrophin isoform deficiency into 5 groups. The cardiac dysfunction (left ventricular ejection fraction <53%)-free survival was significantly higher in the group with mutations in the Dp116 coding region than the others, whereas no significant differences in the other 3 groups. At age 25 years, the cardiac dysfunction-free rate was 0.6 in the Dp116-deficient group, but only 0.1 in others. These results indicate that Dp116 is associated with cardiac involvement in DMD. Our evidence, showing that a lack of Dp116 is associated with improved cardiac function, may provide a mutation-specific cardiac management and a novel therapeutic approach for patients with cardiomyopathy. A drug discovery approach aiming to suppress Dp116 in the human heart should be considered to prevent cardiac dysfunction in patients with DMD.

Duchenne muscular dystrophy (DMD; Online Mendelian Inheritance in Man No. 310200) is the most common inherited muscle disease in childhood, affecting ≈1 of 5000 to 10 000 male newborns.[1] DMD is characterized by fatal progressive muscle wasting. Boys affected by DMD show initial muscle weakness at age 3 to 5 years, with weakness progressing with age and eventually resulting in loss of ambulation by age 12 years. Early death of patients with DMD is caused by wasting of respiratory or cardiac muscles. The life expectancy of patients with DMD has increased, from 15 to 19 to >30 years, through the benefits of multidisciplinary care, especially developments in respiratory care.[2,3] This increase in lifespan, however, has allowed cardiac involvement to emerge as a major cause of morbidity and mortality in patients with DMD.[4–6]

Cardiac involvement, such as dilated cardiomyopathy, is nearly ubiquitous in patients with DMD aged >18 years,[5] with dilated cardiomyopathy being the leading cause of cardiac death in these patients.[7,8] Clinical guidelines recommend early detection and pharmacological intervention to prevent the development of fatal dilated cardiomyopathy.[5,9] For example, an initial echocardiogram is performed at the time of diagnosis or by 6 years of age, with repeat echocardiograms every 1 to 2 years until age 10 years. Older patients have an annual echocardiogram to assess left ventricular (LV) function.[10,11]

DMD is caused by mutations in the *DMD* gene, located on the X chromosome, and is characterized by complete loss of muscle dystrophin, a protein that links the cytoskeleton to the extracellular matrix to form the dystrophin glycoprotein complex. *DMD* is the largest human gene, consisting of 79 exons with at least 8 alternative promoters scattered along the gene. Four promoters at the 5′ end of *DMD* encode unique first exons, producing 4 full-length isoforms (Dp427l, Dp427c, Dp427m, and Dp427p).[12] Four internal promoters, located in introns 29, 44, 55, and 62, encode the short isoforms Dp260, Dp140, Dp116, and Dp71, respectively. Each isoform is expressed in a tissue-specific or development-specific manner, with all isoforms containing a common domain for the formation of the dystrophin glycoprotein complex. Isoform related pathology of DMD has been reported. Dp427m deficiency may occasionally be compensated for by high expression of Dp427c and Dp427p.[13] An X-linked dilated cardiomyopathy, characterized by complete loss of dystrophin expression in cardiac muscle, was found to be accompanied by normal levels of dystrophin expression in skeletal muscle.[14]

Distinct mutations in *DMD* have been reported to correlate with increased incidence of cardiomyopathy or protection against dilated cardiomyopathy.[15] Another study, however, found no correlations between mutation types and cardiomyopathy.[16] These studies focused on the locations of mutations in *DMD*. To our knowledge, the correlation between cardiac involvement in DMD and mutations affecting specific dystrophin isoforms has not been analyzed.

This study on Japanese boys with DMD was designed (1) to clarify the age-related changes on echocardiography and (2) to determine whether cardiac involvement is correlated with dystrophin isoform deficiency.

METHODS

The data, analytic methods, and study materials will be available to other researchers for purposes of reproducing the results or replicating the procedure.[17]

Patients

Study Design and Patient Recruitment

The medical records of patients with DMD registered at the Department of Pediatrics, Kobe University Hospital, located in the western part of Japan, between August 2007 and January 2017, were retrospectively reviewed. The clinical diagnosis of DMD was confirmed by identification of mutations in *DMD* or

immunohistological examination using muscle biopsy samples. Gene mutations were analyzed in both genomic DNA and mRNA extracted from muscle or lymphocytes, as described.[18] The identified mutations met the reading frame rule indicating that each was an out-of-frame or nonsense mutation.[19]

Groupings by Mutation Type and Clinical Findings

The recruited patients with DMD were divided into 5 groups based on mutations affecting dystrophin isoforms. The Dp427 group included all patients with DMD, whereas the Dp260, Dp140, Dp116, and Dp71 groups included patients with mutations in exons 30 to 79, 45 to 79, 56 to 79, and 63 to 79, respectively. Clinical data, included age, height (cm), weight (kg), systolic blood pressure (mm Hg), diastolic blood pressure (mm Hg), heart rate (bpm), LV diastolic dimension (mm) and LV ejection fraction (LVEF, %), and creatine kinase (IU/L), aldolase (IU/L), and brain natriuretic peptide (pg/mL) concentrations, were obtained from hospital records of the day when echocardiographic examination was conducted.

Ethics

The Ethics Committee of the Graduate School of Medicine, Kobe University, approved the present study (approval No. 1534). Informed consent was obtained from either patients or their parents.

Echocardiography

All echocardiograms were obtained by 1 examiner (T. Yamamoto), with considerable experience in imaging of patients with DMD, using a commercially available echocardiographic system (Aplio XG; Toshiba Medical Systems, Tochigi, Japan). Echocardiographic examination of patients with DMD was scheduled annually until age 12 years and biannually thereafter. All patients were recorded in the supine position. Routine digital grayscale 2-dimensional cine loops from 3 consecutive beats were obtained from the parasternal long-axis, short-axis, and standard apical views. As recommended by the American Society of Echocardiography, the LV end-diastolic dimension was obtained using the parasternal long-axis view.[20] The LVEF was assessed by the modified Simpson method, with cardiac dysfunction defined as an LVEF <53%.[20] LV dilation was defined as LV end-diastolic dimension >55 mm.[21] Data from multiple examinations of a single patient were collected at intervals of at least 6 months.

Dp116 Transcript Analysis

Human total RNA from cardiac and skeletal muscles was obtained from a human total RNA Master Panel II (Clontech Laboratories, Inc, Mountain View, CA). cDNA was synthesized from 0.5 µg of each total RNA using random primers as described.[22] The cDNA fragment extending from exon 1 to exon 8 was PCR amplified using the primer set; 1c (5′-ATGCTTTGGTGGGAAGAAGTAG-3′) and c8r (5′-GTAGGACTTCTTATCTGGATA-3′), and the cDNA fragment extending from the Dp116 specific exons 1 to 62 was PCR amplified using the primer set; Dp116ex1F (5′-GGG TTTTCTCAGGATTGCTATGC-3′) and 4F (5′-GAGGAGGTCAA TACTGAGTGGG-3′). Amplifications were performed in a total volume of 10 µL, containing 1 µL cDNA, 1 µL 10× ExTaq buffer (Takara Bio, Inc, Shiga, Japan), 0.25 U ExTaq polymerase (Takara Bio, Inc), 500 nmol/L of each primer, and 250 µmol/L dNTPs (Takara Bio, Inc) on a Mastercycler Gradient PCR machine (Eppendorf AG, Hamburg, Germany). The amplification protocol consisted of an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 3 minutes. Amplified PCR products were electrophoresed using a DNA 1000 LabChip kit on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Reverse transcription-PCR of the *GAPDH* gene (glyceraldehyde 3-phosphate dehydrogenase) was performed as described.[23]

Statistical Analysis

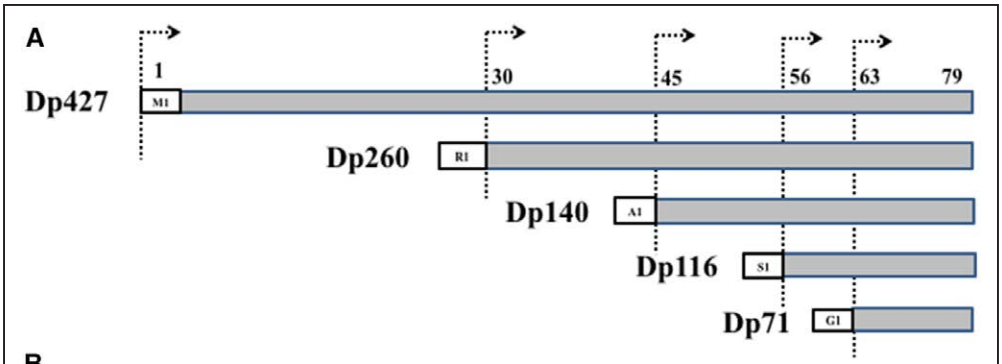
Parameters were expressed as mean±SD or percentage. Patients were stratified into isoform groups, which were compared by multiway ANOVA. The correlation between age and LVEF was analyzed using the mixed effect model. The Kaplan–Meier method was used to estimate survival without cardiac dysfunction and LV dilatation, with groups compared by the log-rank test. DMD groups were compared using Cox proportional hazards models. Differences were evaluated using the χ^2 test or Fisher exact test, as appropriate. In all analysis, P values <0.05 were considered statistically significant. All analyses were performed with commercially available software R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient's Characteristics

A total of 292 consecutive patients with DMD were enrolled at our center between August 2007 and January 2017. Of these, 109 were excluded because they underwent regular echocardiographic examinations at local hospitals. Two additional patients were also excluded, one with a chromosomal abnormality and the other not verified as having DMD at the genomic DNA level. Thus, this study included 181 patients. Their mean age at initial echocardiographic evaluation was 10.1±4.6 years (range, 4 to 25 years). Genetically, the most common types of mutations were deletions/duplications of one or more *DMD* exons, with deletions observed in 103 (57%) and duplications in 15 (8%) of the 181 patients (Figure I in the Data Supplement). The second most common type of mutation was nonsense mutations, observed in 38 (21%) patients. Mutations identified in the remaining patients included small insertions/deletions, splice site mutations, and deep intron mutations (Figure I in the Data Supplement). This cohort showed no particular predisposition to any specific mutation.

To analyze the correlations between cardiac involvement and dystrophin isoform deficiency, the 181 patients with DMD were divided into 5 groups based on identified mutations in *DMD* (Table I in the Data Supplement). The Dp427 group, consisting of all 181 patients, was characterized by mutations at any position in *DMD*. The Dp260, Dp140, Dp116, and Dp71 groups consisted of 135, 116, 21, and 12 patients, respectively (Figure 1). There were no significant differences among the groups



Isoform	Total (n)	Analyzed* (n)
Dp427	181	162
Dp260	135	120
Dp140	116	102
Dp116	21	19
Dp71	12	11

Figure 1. Grouping of patients with Duchenne muscular dystrophy (DMD).
A, The entire coding region of each dystrophin isoform is schematically described. Open and shaded boxes represent tissue-specific exon 1 and DMD exons, respectively. Numbers over boxes indicate exon number. **B**, All 181 patients with DMD belonged to the Dp427 group. Patients in the Dp260 (n=135), Dp140 (n=116), Dp116 (n=21), and Dp71 (n=12) groups had mutations in exons 30 to 79, 45 to 79, 56 to 79, and 63 to 79, respectively. *Numbers of patients who were analyzed for the Kaplan–Meier method.

in age, height, weight, systolic blood pressure, diastolic blood pressure, heart rate, LV diastolic dimension, LVEF, and concentrations of creatine kinase, aldolase, and brain natriuretic peptide, when clinical data obtained at the first echocardiographic examination were compared (Table II in the Data Supplement). Although not statistically significant, serum creatine kinase and aldolase concentrations were highest in the Dp116 group, whereas age, height, systolic and diastolic blood pressure, LV diastolic dimension, and LVEF were lowest in this group.

Changes Over Time in LVEF

The mean follow-up from initial echocardiographic evaluation was 5.0±2.6 years (range, 0.7 to 9.4 years). A review of their medical records identified 1109 echocardiograms from the 181 recruited patients with DMD. On average, individual patients underwent 6 echocardiographic examinations, with 1 patient undergoing 16 examinations. Overall, LVEF declined with age from 4 to 33 years (Figure 2A), with the 2 being significantly correlated (r=–0.806; P<0.001) by the mixed effect model, indicating an aging-dependent decrease in LVEF. The annual change in LVEF was evaluated by calculating the mean LVEF values every 1 year (Figure 2B). LVEF remained at >65% until patients reached an age of 8 years. Thereafter, LVEF started to decrease gradually, with the decreased becoming sharper after age 11 years, reaching a plateau of 40% to 50% at age 15 to 25 (Figure 2B). Beginning at age 26 years, LVEF began to decrease sharply over time, indicating a stepwise decrease in LVEF.

Cardiac Dysfunction-Free Survival

Cardiac dysfunction (LVEF <53%) is the major outcome of dystrophin-deficient cardiomyopathy. Cardiac

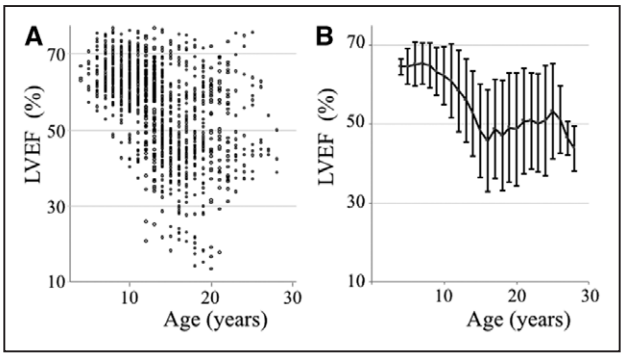


Figure 2. Changes in left ventricular ejection fraction (LVEF) over time in patients with Duchenne muscular dystrophy.
A, Individual LVEF values at 1-y intervals from ages 4 to 33 y. LVEF significantly declined with age (r=–0.806; P<0.001). **B**, Mean±SD LVEF values at 1-y intervals.

dysfunction-free survival was estimated by the Kaplan–Meier method. For this analysis, 19 patients who were already in cardiac dysfunction at the first examination were excluded (Figure 1B). As a result, the remaining 162 patients were analyzed by the Kaplan–Meier method (Figure 3). At age 14 years, cardiac dysfunction was observed in nearly half of the patients. Up to age 7 years, no patient showed cardiac dysfunction. Cardiac dysfunction-free survival started to decrease from age 8 years when one patient showed cardiac dysfunction. A sharp decline in the survival rate was observed at 14 years of age. At 25 years of age, only 20% of patients were cardiac dysfunction-free survivors.

Less Frequent Cardiac Dysfunction in the Dp116 Than in the Others

To explore the effects of dystrophin isoform on cardiac dysfunction, cardiac dysfunction-free survival rates were estimated by the Kaplan–Meier method in 5 DMD patient groups. However, apparent difference was not disclosed in their curves (Figure II in the Data Supplement). In this grouping, some patients grouped into >2 groups. To avoid this overlapping, each isoform deficient group was compared with others who expressed the respective isoform (Figure 4). The survival rate of the Dp260 group (n=120) was similar to that in the others (n=42), with both having a median age for cardiac dysfunction of 15 years and no significant between-group difference by the log-rank test (Figure 4A). Furthermore, cardiac dysfunction-free survival rates in the Dp140 (n=102) and Dp71 (n=11) groups did not differ significantly from in the others (Figure 4B and 4D). In contrast, the cardiac dysfunction-free survival rate was significantly higher in the Dp116 group (n=19) than in the other patients (n=143; log-rank test, *P*=0.022; Figure 4C). At age 25 years, the survival rate was 0.6 in the Dp116 group, and 0.15 in the other patients. Moreover, patients in the Dp116 group were at lower risk of cardiac dysfunction than the other patients (hazard ratio, 2.89; 95% confidence interval, 1.038–8.045; *P*=0.042). Although this difference may have been because of a greater use of cardioprotective medications in the Dp116 group, there were no significant differences in treatment with ACE inhibitors, β-blockers, and steroids at the time of the last echocardiographic examination (Table), suggesting that cardiac dysfunction is less frequent in Dp116 than in other patients with DMD.

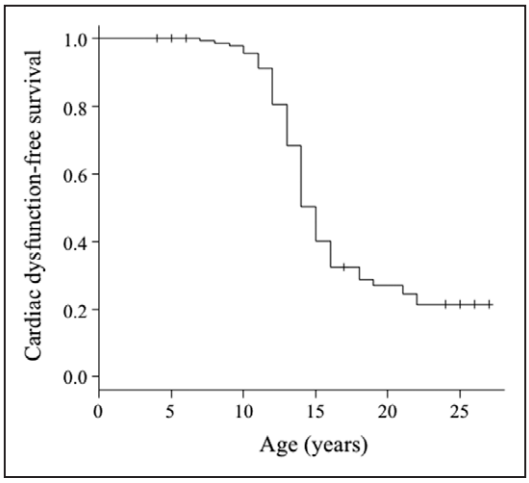


Figure 3. Cardiac dysfunction-free survival of 162 patients with Duchenne muscular dystrophy (DMD).
The median age of overall survival without cardiac dysfunction (<53%) was 14 y.

LV Dilation-Free Survival

LV dilation (LV end-diastolic dimension >55 mm)-free survival was also estimated by the Kaplan–Meier curve (Figure 5). The survival rate was 0.85 at 25 years. No DMD patient showed LV dilation until age 12 years, followed by a decline until age 17 years.

LV Dilation-Free Survival in the 4 DMD Groups

LV dilation-free survival rates were estimated in the various DMD patient groups and compared with those in other patients (Figure 6). None of these comparisons showed significant differences, including a comparison of patients in the Dp116 group with all other patients (log-rank test, *P*=0.23).

Dp116 in Human Cardiac Muscle

The above analysis indicated that the Dp116 deficiency when added to the Dp427 deficiency, present in all patients, protects against cardiac dysfunction in DMD. Biologically, however, Dp116 expression had not been detected in human cardiac muscle. The 5′ end region of Dp116 cDNA (exon S1 to 62) from both cardiac and skeletal muscles was therefore PCR amplified, yielding a product of expected size from both samples (Figure 7). Sequencing of these products confirmed their identity as being the 5′ end of Dp116 mRNA (data not shown). The amplified product from cardiac muscle was more abundant than that from skeletal muscle, indicating that Dp116 mRNA was expressed in cardiac muscle.

DISCUSSION

Cardiomyopathy is the most important nonmuscle symptom threatening the life of patients with DMD.[5,6] Many studies, using techniques such as electrocardiography,[24,25] echocardiography,[25–27] and MRI,[26,28] have been performed to understand the pathophysiology of the dystrophin-deficient heart.

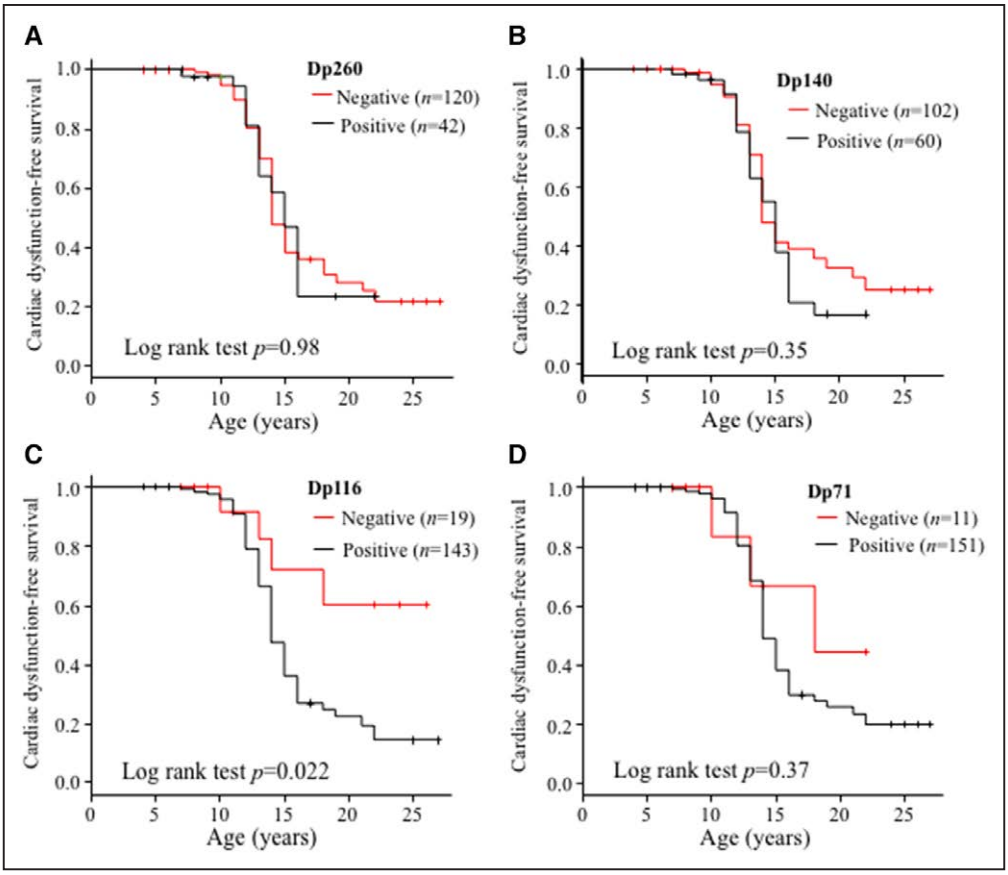


Figure 4. Cardiac dysfunction-free survival.
Survival curves were calculated using the Kaplan–Meier method and analyzed by the log-rank test. **A**, Dp260 group (negative) vs all other patients (positive; log-rank test, *P*=0.98). **B**, Dp140 group vs all other patients (log-rank test, *P*=0.35). **C**, Dp116 group vs all other patients (log-rank test, *P*=0.022). **D**, Dp71 group vs all other patients (log-rank test, *P*=0.37).

In the present study, echocardiography was used to assess LVEF and LV end-diastolic dimension in 181 patients with DMD. The 1109 echocardiograms obtained from these patients showed that LVEF naturally declined with age, as reported previously.[7] We observed sharp declines in LVEF at ages 11 and 26 years, indicating a stepwise deterioration of cardiac function in DMD. Age 11 years is a critical point for prevention of cardiomyopathy, with clinical guidelines recommending an annual echocardiographic examination after age 10 years.[10,11]

Genotype–phenotype correlations in dystrophin-deficient cardiomyopathy have long been the subject of DMD studies. Mutations involving exons 12, 14 to 17, 31 to 42, 45, and 48 to 49 have been reported to enhance cardiac involvement.[7,15,29–33] In contrast, mutations in exons 51 to 52 were shown to be protective against cardiac involvement.[15] Another study, however, failed to identify associations between mutations and LV dysfunction.[16] Searches for genetic modifiers of dilated cardiomyopathy in patients with DMD indicated that the dominant G allele of the osteopontin gene and the recessive T allele of the latent TGF-β (transforming growth factor-β)–binding protein 4 gene tended to have protective effects.[34] Surprisingly, the present study found that the cardiac dysfunction-free survival rate was significantly higher in DMD patients with than without mutations in the Dp116 coding region. This significant difference was not caused by differences in medications. Rather, the deficiency of Dp116, when added to the deficiency of Dp427, present in the entire study cohort, may protect against cardiac dysfunction.

However, Dp116 expression in the human heart had not been determined. Dp116 is a nonmuscle isoform of dystrophin involved in the assembly of dystrophin glycoprotein complexes, but lacking actin-binding

Table. Number of Patients With Pharmacological Intervention Among Duchenne Muscular Dystrophy Groups

Medicine	Dp260	Dp140	Dp116	Dp71	*P* Value
ACEi	45 (38)	39 (38)	5 (26)	4 (36)	0.82
β-blocker	44 (37)	39 (38)	5 (26)	4 (36)	0.81
Steroid	38 (32)	34 (33)	6 (32)	2 (18)	0.85

Numbers and numbers in the parenthesis indicate the number of treated patient's and its percentage, respectively. ACEi indicates angiotensin-converting enzyme inhibitor.

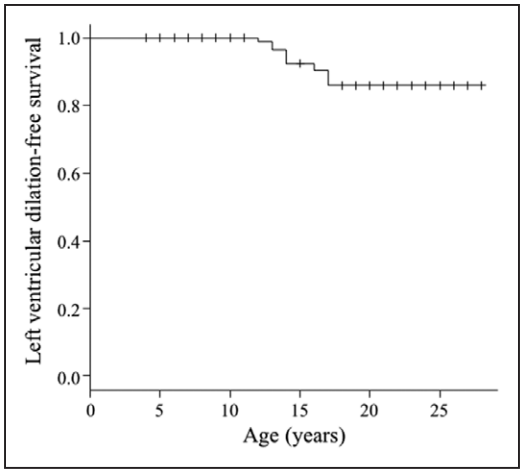


Figure 5. Left ventricular (LV) dilation (LV end-diastolic dimension >55 mm)-free survival.
The survival rate at 25 y was 0.85.

domains. Expression of Dp116 was observed in the peripheral nerves and brain,[35] but not in mouse and monkey hearts.[35,36] RT-PCR, however, showed Dp116 mRNA in mouse hearts.[37] Few studies have analyzed Dp116 expression in humans, with most focusing on its role in Schwann cells.[35,38,39] We observed Dp116 mRNA expression in human heart samples, substantiating our echocardiographic finding that Dp116 promotes cardiac damage in patients with DMD.

The present results suggest that Dp116 expression was pathogenic, but not compensatory, for Dp427 deficiency. These findings are compatible with results showing that the dystrophin C-terminal fragment was sufficient to cause marked dystrophic cardiomyopathy in mice.[40] However, a study in *Drosophila* found that introduction of mouse Dp116 into the hearts of *dys* mutant flies rescued the dilated cardiomyopathy phenotype.[41] This finding conflicted with our results showing that the Dp116 deficiency resulted in better outcomes for cardiac involvement. Future studies are needed to address this discrepancy.

Although the Dp116 group showed better outcomes for cardiac dysfunction, it did not show better outcomes for LV dilation. The absence of Dp427 may be sufficient to induce LV dilation because the size determination linking cytosolic actin and transmembrane β-dystroglycan is lost.[5] The development of cardiac dys-

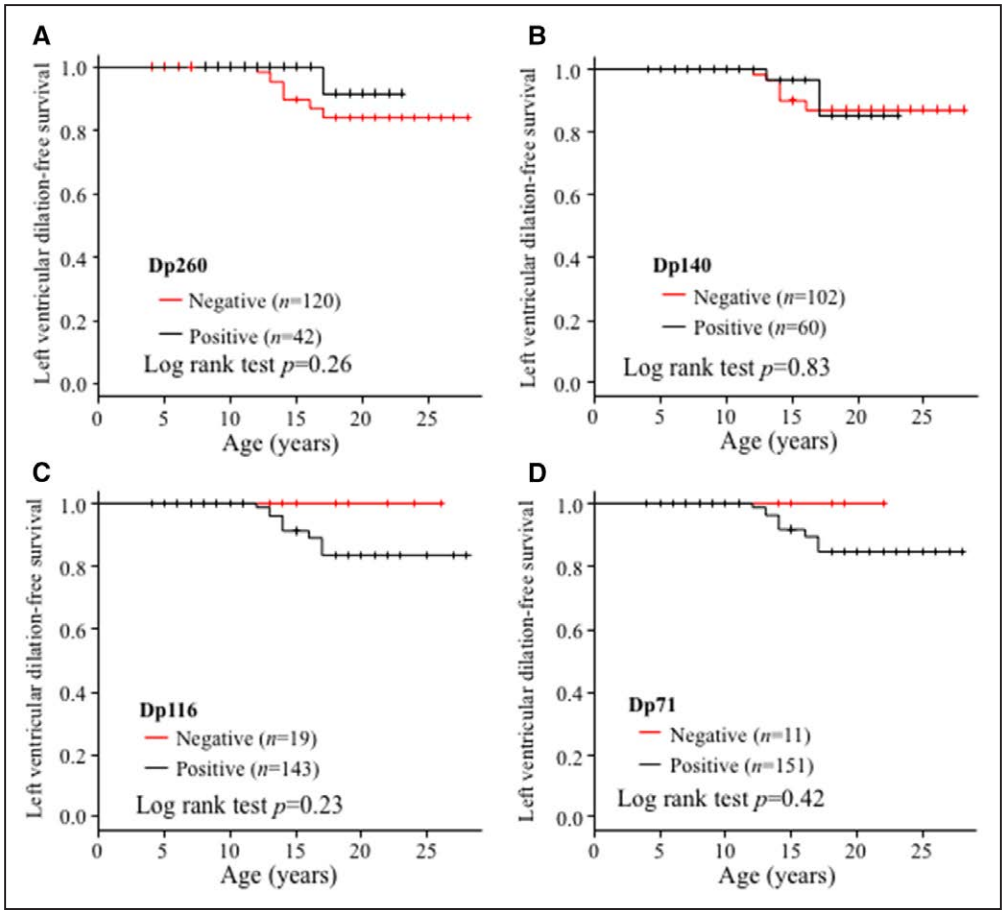


Figure 6. Left ventricular dilation-free survival.
Survival curves were calculated using the Kaplan–Meier method and compared by the log-rank test. **A**, Dp260 group (negative) vs all other patients (positive). **B**, Dp140 group vs all other patients. **C**, Dp116 group vs all other patients. **D**, Dp71 group vs all other patients. None of these differences was statistically significant.

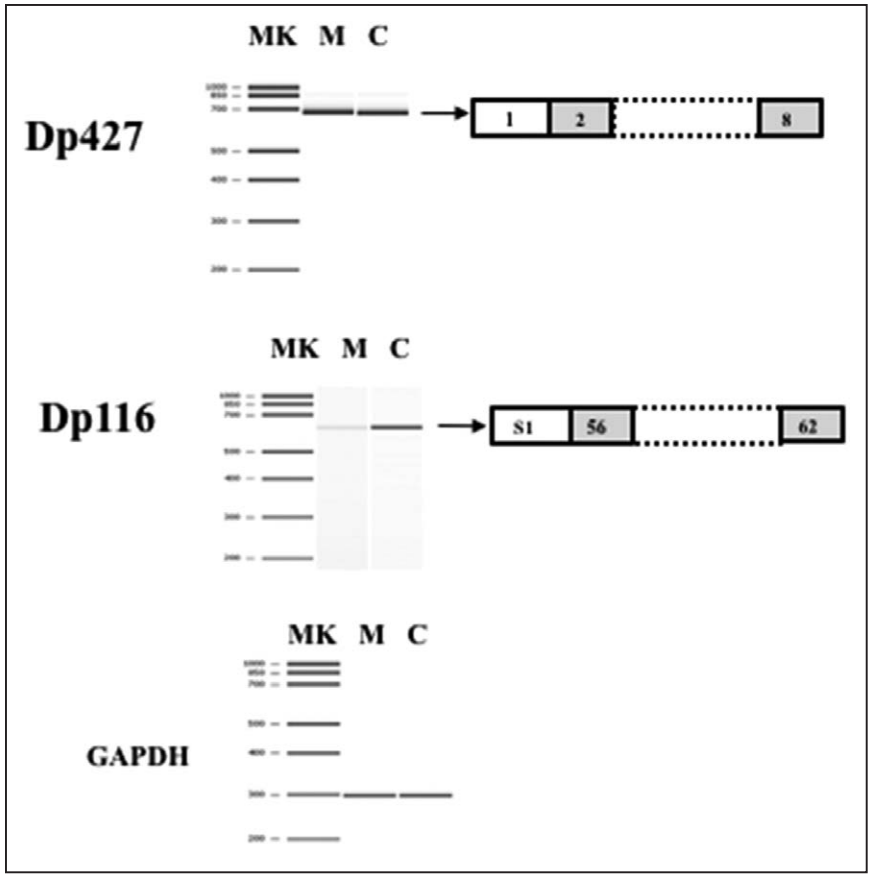


Figure 7. Characterization of the Dp116 transcript in cardiac muscle.
The 5′ end of Dp427 and Dp116 transcript was amplified as a fragment extending from exon 1 to exon 8 and from exon S1 to exon 62, respectively. Electropherograms of the amplified product are shown. One clear amplified product of Dp116 transcript was obtained from cardiac (C) and skeletal (M) muscles (Dp116). The exon structure is schematically described on the right. Boxes and numbers in the boxes indicate exons and exon numbers, respectively. As an internal standard, GAPDH transcript was amplified (GAPDH).

function may require both Dp116 and Dp427 deficiency. New cardiac-specific dystrophin-associated proteins have recently been identified.[42] The presence of Dp116 provides a scaffold for the formation of cardiac-specific dystrophin-dystroglycan complexes that play important functional roles leading to cardiac dysfunction.

Our evidence, showing that a lack of Dp116 is associated with improved cardiac function, may provide a novel therapeutic approach for patients with cardiomyopathy. Suppression of Dp116 expression may prevent cardiac dysfunction in patients with DMD. A drug discovery approach aiming to suppress Dp116 in the human heart should be considered.

This was a nonrandomized, retrospective, observational single-center study with all the inherent limitations of such a study. Our results were obtained from routine clinical echocardiographic examinations. The feasibility and reproducibility of echocardiographic measures in patients with DMD have been confirmed.[43] Recent studies, however, have examined cardiac dysfunction using tissue Doppler echocardiography or cardiovascular MRI.[26,28,44–46] It will be interesting to clarify the genotype–phenotype correlations based on cardiovascular magnetic resonance results. Another potential limitation of this study is the small number of patients, but it is difficult to enroll a large cohort of patients with a rare disorder such as DMD.

CONCLUSIONS

LVEF in DMD declined in a stepwise pattern by age. DMD patients with mutations in the Dp116 coding region showed better outcomes for cardiac dysfunction. The present findings suggest a new therapeutic target involving disruption of Dp116 in patients with DMD.

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DISCLOSURES

Dr Matsuo is an advisor for JCR Pharma Co, Japan, and Daiichi Sankyo Co Ltd, Japan. The other authors report no conflicts.

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FOOTNOTES

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The Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGEN.117.001782/-/DC1.

An educational video is available at http://circgenetics.ahajournals.org/highwire/filestream/257350/field_highwire_adjunct_files/1/CircGenetics_CIRCCVG-2018-001782_supp11.mp4.

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