The ACTN3 577XX Null Genotype Is Associated with Low Left Ventricular Dilation-Free Survival Rate in Patients with Duchenne Muscular Dystrophy

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Full title: The *ACTN3* 577XX null genotype is associated with low left ventricular dilation-free survival rate in Duchenne muscular dystrophy patients

ACTN3 genotype relates cardiomyopathy in DMD

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Abbreviations

DMD, Duchene muscular dystrophy; LV, left ventricular; DCM, Dilated cardiomyopathy; ACE, angiotensin-converting enzyme; EF, Ejection fraction; LVEF, Left ventricular ejection fraction; RFLP, Restriction fragment length polymorphism; ZASP, Z-disc-associated alternatively spliced PDZ motif-containing protein; PCR, Polymerase chain reaction; RFLP, Restriction fragment length polymorphism

Abstract

Background

Duchenne muscular dystrophy (DMD) is a fatal progressive muscle-wasting disease caused by mutations in the *DMD* gene. Dilated cardiomyopathy is the leading cause of death in DMD; therefore, further understanding of this complication is essential to reduce morbidity and mortality.

Methods

A common null variant (R577X) in the *ACTN3* gene, which encodes α-actinin-3, has been studied in association with muscle function in healthy individuals, however not yet examined with cardiac phenotype in DMD. Here, we determined the *ACTN3* genotype in 163 DMD patients and examined the correlation between *ACTN3* genotypes and echocardiographic findings in 77 of the 163 patients.

Results

The genotypes 577RR(RR), 577RX(RX), and 577XX(XX) were identified in 13 (17%), 44 (57%), and 20 (26%) of 77 patients, respectively. We estimated cardiac involvement-free survival rate analyses using Kaplan-Meier curves. Remarkably, the left ventricular (LV) dilation (LVDd>55 mm)-free survival rate was significantly lower in XX null genotype patients (P<0.01). XX null genotype showed a higher risk for LV dilation (hazard ratio 9.04).

Conclusions

This study revealed that *ACTN3* XX null genotype was associated with a lower LV dilation-free

survival rate in DMD. These results suggest that *ACTN3* genotype should be determined at the time of diagnosis of DMD to improve patients' cardiac outcomes.

Keywords: Duchenne muscular dystrophy; alpha-actinin 3; dilated cardiomyopathy

Introduction

Duchenne muscular dystrophy (DMD; MIM No.310200) is the most common inherited muscle disease, estimated to affect approximately 1 out of 3,500 to 5,000 male newborns.[1,2] It is a fatal disease diagnosed in childhood and characterized by progressive muscle wasting caused by a mutation in the *DMD* gene. Affected individuals commonly lose their ability to walk around 10 years after beginning to walk in infancy.[3,4] Patients with DMD have multiple complications, of which cardiomyopathy and resultant heart failure is the most common cause of morbidity and mortality.[5,6] The incidence of cardiomyopathy increases with age in DMD. Although it is estimated that 25% of boys have cardiomyopathy at 6 years of age, an incidence of cardiac involvement becomes ubiquitous, reaching more than 90%, in patients aged over 18.[7] Left ventricular (LV) dilation is major feature of progressive cardiomyopathy, and is evident in patients starting in their early teenage years through to their twenties.[8,9] As a consequence of LV dilation, cardiomyopathy manifests as a dilated cardiomyopathy (DCM) resulting in heart failure and cardiac death.[10,11] Therefore, DCM is the end result of cardiac involvement in DMD. To prevent progression to DCM, clinical guidelines recommend cardiac evaluation using echocardiography and electrocardiography in all patients with DMD. Current recommendations call for initial cardiac evaluation starting from the time of diagnosis or at 6 to 7 years of age, then every 1 to 2 years until 10 years of age, and more frequently thereafter in patients with evidence of cardiac involvement.[5,12] Once patients with DMD have features of cardiac

impairment or reduced ejection fraction (EF), early intervention including angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, and beta-blockers are recommended. Although the management of cardiac complications in patients with DMD has evolved over the past several decades, cardiac involvement still represents a major obstacle impacting survival, therefore; further research into this important complication is warranted.

The *ACTN3* gene encodes α-actinin-3, one of the major structural components of sarcomeric Z-discs.[13] There is a common null variant, c.1729C>T (p.R577X) (rs1815739) (NM_001104.4), in the gene coding for α-actinin-3. The frequency of the p.R577X ranges from 10% to 70% worldwide,[14,15] with homozygous carriers of the variant having a complete deficiency of α-actinin-3. In fact, western blot analysis using human skeletal muscles revealed reduced and no detectable expression of α-actinin-3 in individuals with p.577R/p.577X genotype and homozygous p.577X genotype.[16] This common null variant has been reported to be associated with reduced muscle strength in the general population.[17,18] Despite the association with possible muscle dysfunction, the correlation between *ACTN3* genotype and DMD phenotype has not been thoroughly investigated. Suminaga *et al.*[19] reported that the genotype was not associated with the age at which DMD patients first walked. Hogarth *et al*.[20] found that the genotype is associated with isolated muscle strength and overall muscle function in patients with DMD. These studies introduced the hypothesis that *ACTN3* is a disease modifier for DMD. Recently, *ACTN3* genotype has been reported to be correlated with survival in

patients with chronic heart failure,[21] suggesting an association between *ACTN3* genotype and cardiac function.

This study was designed to determine whether cardiac involvement is related to the *ACTN3* genotype in Japanese boys with DMD.

Methods

This is a single-center retrospective study. DMD patients were registered at the Department of Pediatrics, Kobe University Hospital between June 1992 and March 2018. The medical records of registered patients with DMD were retrospectively reviewed. The clinical diagnosis of DMD was confirmed by the identification of mutations in *DMD*. Gene mutations were analyzed in both genomic DNA and mRNA extracted from the muscle or peripheral lymphocytes, as previously described.[22] The reading frame rule including out-of-frame and nonsense mutation was applied to diagnose DMD.[23] To examine the correlation between cardiac function and *ACTN3* genotype by the Kaplan-Meier method, this study was conducted in patients who fulfilled the following inclusion criteria: 1) DMD patients whose genomic DNA was conserved in our laboratory; 2) who underwent routine echocardiography in our hospital, and 3) who did not show any echocardiographic findings indicating cardiac dysfunction or dilated cardiomyopathy at the first examination in our hospital.

Peripheral blood samples were obtained from patients. Genomic DNA was isolated using standard

phenol-chloroform extraction methods. *ACTN3* exon 15 was amplified by polymerase chain reaction (PCR) using the forward primer ACTN3-E15F (5'-CGCCCTTCAACAACTGGCTGGA-3') and reverse primer ACTN3-E16R (5'-GGGTGATGTAGGGATTGGTGGAG-3'). Thirty-five PCR cycles were performed in a mixture of 1.0 μL gDNA, 2.0 μL of 10 ExTaq buffer (Takara Bio, Inc, Shiga, Japan), 0.5 U of ExTaq polymerase (Takara Bio, Inc), 0.5 μL of 10 μmol/L of each primer, and 2.0 μL of 250 μmol/L dNTPs (Takara Bio, Inc) using the following conditions: initial denaturation at 96 °C for 1 min, subsequent denaturation at 96 °C for 0.5 min, annealing at 62 °C for 0.5 min and extension at 72 °C for 1 min. The final extension reaction was carried out at 72 °C for 5 min. The purified PCR-amplified products were sequenced using the Premix sequencing system by Fasmac Co., Ltd. (Kanagawa, Japan).

All echocardiograms were obtained by one examiner (T. Yamamoto), with considerable experience in the imaging of patients with DMD, using a commercially available echocardiographic system (Aplio XG; Canon Medical System Corporation, Tochigi, Japan). Echocardiographic examination of patients with DMD was scheduled annually until the age of 12 years and biannually thereafter. Routine digital grayscale 2-dimensional cine loops from 3 consecutive beats were obtained from the parasternal long-axis, short-axis, and standard apical views. Following the American Society of Echocardiography recommendations, the LV end-diastolic dimension was obtained using the parasternal long-axis view.[24] LV-dilation was defined as LV end-diastolic dimension >55 mm. The LV ejection fraction (EF) was

assessed by the modified Simpson method and cardiac dysfunction was defined as an LVEF <53%.[24] Data from multiple examinations of a single patient were collected at an interval of at least 6 months.

The Shapiro-Wilk test was used to determine if a data set was modeled by a normal distribution. When the P-value was <0.05, the data set was considered to be in a non-normal distribution. The Student's *t*-test or ANOVA were used to compare findings between two groups or among three groups, respectively, if the data were distributed normally; otherwise, the Mann-Whitney *U* test or Kruskal-Wallis test were used. Differences were evaluated using Fisher's exact probability for comparing categorical measures between groups (oral prednisolone use, ACE inhibitor use and beta-blocker use). The Kaplan-Meier method was used to estimate survival without cardiac dysfunction, LV dilation and loss of ambulation, with groups compared by the log-rank. The Mantel-Haenszel test was used to estimate the hazard ratio between two groups. In all analyses, P values <0.05 were considered statistically significant. All analyses were performed with commercially available software GraphPad Prism 7.02 (GraphPad Software, Inc, San Diego, CA).

The study protocol was approved by the ethics committee of the Graduate School of Medicine, Kobe University (Approval No.180356). This study was conducted in compliance with the Declaration of Helsinki and the Ethical Guidelines for Human Genome and Gene Analysis Research.

Results

Four hundred and fifty-two patients were diagnosed with DMD and registered in our hospital. Genomic DNA from 163 DMD patients was available for *ACTN3* variant analysis. Of 163 patients, 74 patients were excluded because they underwent routine echocardiographic examinations at local hospitals. In addition, 12 patients had evidence of cardiac dysfunction at the time of first echocardiography, and they were excluded because the onset age of cardiac involvement was unknown. A total of 77 patients were included in the study.

Genetically, the most common types of mutations in the *DMD* gene were deletions/duplications of one or more exons, with deletions observed in 36 (46.8%) and duplications in 7 (9.1%) of the 77 patients (Supplementary Table 1). The second most common type of mutation was a nonsense mutation observed in 20 (26.0%) patients. Mutations identified in the remaining patients were 11 small insertions/deletions, 2 splice site mutations, and 1 deep intron mutation.

Genotyping was completed by direct sequencing in a total of 163 DMD patients including 77 patients who satisfied the inclusion criteria. When patients had a wild type, c.1729C (p.577R), variant in two alleles, *ACTN3* genotype was defined as 577RR (RR). When patients had a single nucleotide variant of c.1729C>T (p. R577X) in one allele, *ACTN3* genotype was defined as 577RX (RX) and in two alleles, as 577XX (XX) (Figure 1a). Genotypes RR, RX, and XX were identified in 31 (19%), 84 (52%), and 48 (29%) of the 163 patients, respectively. The allele frequency of R577X was 0.552. The distribution of genotypes was in accordance with the Hardy-Weinberg equilibrium (X^2=0.29, p=0.87).

Among the 77 patients included in this study, the genotypes RR, RX, and XX were identified in 13 (17%), 44 (57%), and 20 (26%) patients, respectively (Figure 1b). The allele frequency of R577X was 0.545. The distribution of genotypes was in Hardy-Weinberg equilibrium (X^2=1.79, p=0.41).

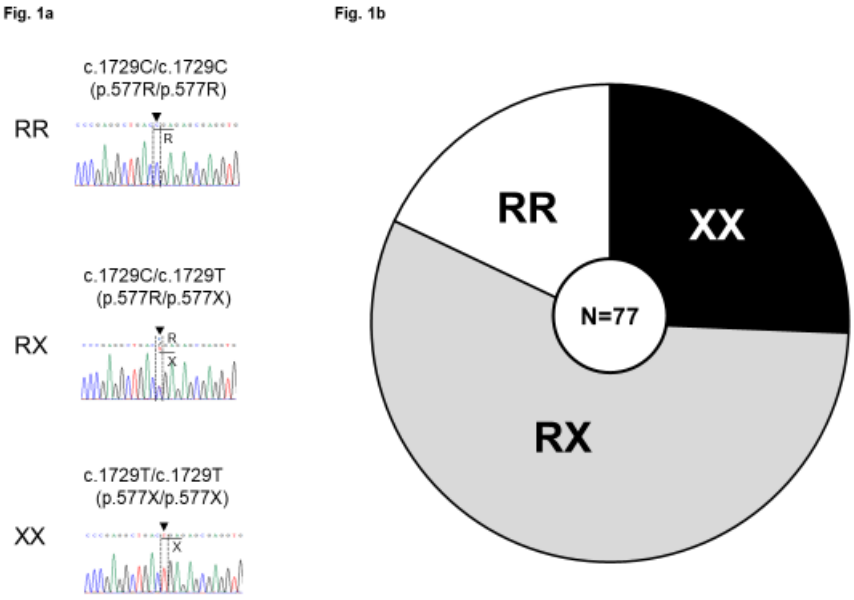

Figure 1. a) Panels show part of the sequence of *ACTN3* exon 15. Upper, middle, and lower panels show the homozygous c.1729C (p.577R) (RR), c.1279C/c.1279T(p.577R/p.577X) (RX), and homozygous c.1279T(p.577X) (XX). Underlines and upper-case alphabetic characters show 577th codons of *ACTN3* and translated amino acids. b) The proportion of the *ACTN3* genotype. RR [13 (17%)], RX [44 (57%)], and XX [20 (26%)] represent *ACTN3* 577RR, 577RX, and 577XX genotype, respectively.

To examine whether there were extraneous factors that may affect cardiac function, clinical characteristics were compared among the different *ACTN3* genotypes (Table 1).

There were no significant differences among the genotypes in initial echocardiographic examination age, final echocardiographic age, oral prednisolone use, ACE inhibitor use, and beta-blocker use. There was also no difference in age at loss of ambulation among the different genotypes. The mean ± SD LVEFs using initial echocardiography data of 577R, 577RX, and 577XX were 67.4±5.3%, 65.2±6.1% and 65.0±5.3%, respectively. There was no significant difference among 3 genotypes (p=0.45). The mean ± SD LVDds of 577R, 577RX and 577XX were 37.6±3.0%, 37.9±4.2% and 39.6±6.1%, respectively, with no statistical difference (p=0.76).

Next, to clarify the effect of α-actinin-3 protein deficiency on cardiac function, we divided the 77 DMD patients into two groups. Considering that the XX group was supposed to be deficient in α-actinin-3,[16] the XX genotype was defined as the *ACTN3* null genotype (N=20). Groups RR and RX were defined as the *ACTN3* positive genotype (N=57). As shown in Table 2, there were no significant differences among the aforementioned characteristics between the groups of different genotypes.

Cardiac dysfunction (LVEF<53%) is the main consequence of cardiomyopathy in DMD; therefore, we first estimated cardiac dysfunction-free survival in the RR, RX, and XX groups (Figure 2a). When the survival rate was compared, there were no significant differences among the three groups (log-

rank test, p=0.103). Subsequently, we compared the cardiac dysfunction-free survival rate between the *ACTN3* null, and positive genotypes (Figure 2b). The median age of cardiac dysfunction-free survival in the *ACTN3* null, and positive genotypes was 13.4 and 15.3 years, respectively. The cardiac dysfunction-free survival rate in the *ACTN3* null genotype was significantly lower than that in the *ACTN3* positive genotype (log-rank test, p=0.041). At the age of 15 years, the survival rate was only 0.13 in patients with the *ACTN3* null genotype compared to 0.45 in *ACTN3* positive genotype patients. The *ACTN3* null genotype was significantly associated with earlier onset of cardiac dysfunction compared to the other genotypes, and showed a higher risk for cardiac dysfunction with a hazard ratio of 2.78 (95% confidence interval, 1.04 – 7.44).

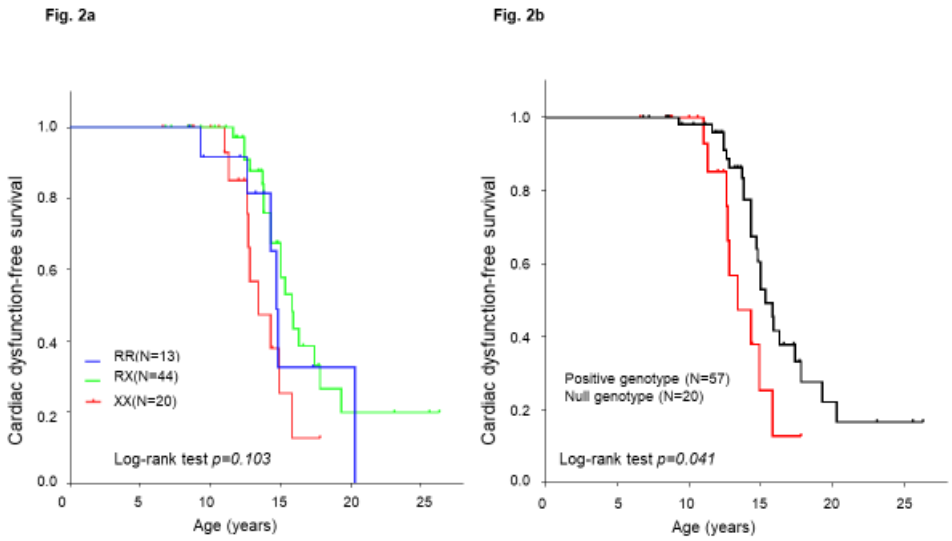

Figure 2. Cardiac dysfunction-free survival. Survival curves were calculated using the Kaplan-Meier method and were analyzed by the log-rank test. A) Cardiac dysfunction-free survival in patients

with RR (blue), RX (green), and XX genotype (red). B) Cardiac dysfunction-free survival in patients with *ACTN3* positive genotype (black), and *ACTN3* null genotype (red).

Progressive LV enlargement underlies the pathophysiology of cardiomyopathy.[8,9] To better understand this, we estimated LV dilation (LV end-diastolic dimension >55 mm)-free survival using the Kaplan-Meier curve. Comparison of survival rate in the three genotypes (RR vs RX vs XX) revealed that the XX genotype was significantly associated with early onset of LV dilation (log-rank test, p=0.023) (Figure 3a). LV dilation was not observed in any patients until the age of 12 years; however, patients with the XX genotype showed a fall in the LV dilation-free survival rate from the age of 13 years. At the age of 20 years, LV dilation-free survival rate was only 0.29 in patients with the XX genotype. However, the RX genotype showed a higher LV dilation-free survival rate of 0.77 at the age of 20 years. Surprisingly, no patients with the RR genotype showed LV dilation up to the age of 21 years.

A comparison of the LV dilation-free survival rate between the *ACTN3* null genotype and *ACTN3* positive genotype clarified the role of α-actinin-3 deficiency on the progression of LV dilation (Figure 3b). LV dilation-free rate was significantly lower in the *ACTN3* null genotype than the *ACTN3* positive genotype (log-rank test, p=0.007). Patients with *ACTN3* null genotype had a significant risk for LV dilation (hazard ratio 9.04, 95% confidence interval, 1.77-46.20).

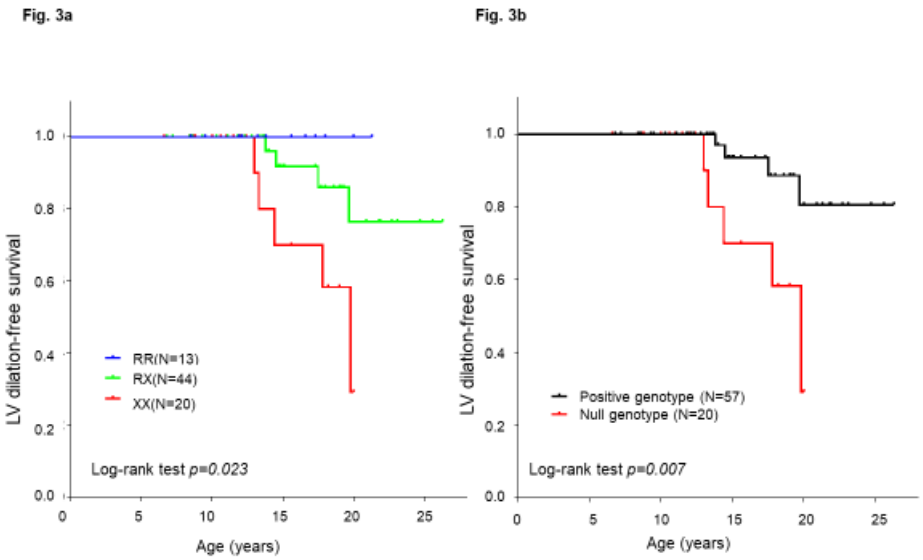

Figure 3. LV dilation-free survival. A) LV dilation-free survival in patients with RR (blue), RX, (green), and XX genotype (red). B) The black and red curve represented the survival rates of the patients with *ACTN3* positive genotype, and *ACTN3* null genotype.

Finally, we compared cardiac dysfunction- and LV dilation-free survival ratio between the wild type (RR genotype) and others (RX and XX genotype) to see the effect of losing one R allele on the cardiac outcome. The aforementioned characteristics between the wild type and others were not significantly different (data are not shown). Both cardiac dysfunction- and LV dilation-free survival ratio were not different (log-rank test, p=0.83 and p=0.19, respectively). These indicated that only the null genotype is associated with worse cardiac survival in DMD.

Discussion

This study found an association between *ACTN3* 577XX null genotype and early progression of DCM in DMD. In the present study, *ACTN3* genotype was determined in the 77 patients with DMD and cardiac involvement-free survival rate was compared among genotypes. We observed a significantly earlier progression to LV dilation in patients with *ACTN3* null genotype.

We determined the *ACTN3* genotype of 163 enrolled patients and the allele frequency of R577X was 0.552. The R577X allele frequency of 77 patients included in this study was 0.545. This allele frequency was similar to the reported frequency in the East Asian population, which ranges from 0.458 to 0.567.[15,19,25] Taken together, selection bias is considered to be eliminated.

In this study, we used direct sequencing to determine *ACTN3* genotype. Single nucleotide variants in the *ACTN3* gene have been identified by restriction fragment length polymorphism (RFLP) or novel high-throughput methods.[19-21] RFLP is a well-established and frequently used method; however, due to its indirect approach, it sometimes fails to accurately establish a genotype.[26] Recent advances in genotyping technology allowed for increased throughput, reduced time and expense, and detection of multiple single nucleotide variants of interest.[27] Nevertheless, direct sequencing remains the preferred method in cases in which the number of markers required is not very large and in studies with limited budgets.[28] Overall, direct sequencing is considered an easy, accurate and cost-effective method for determining the *ACTN3* genotype in patients with DMD.

The *ACTN3* gene encodes α-actinin-3 which is a major component of the Z-disc that defines the lateral border of the sarcomere; consequently, many studies to date have focused on its expression and function in skeletal muscles.[13,29] However, α-actinin-3 is expressed in tissues of the cardiovascular system including human fetal and adult heart,[30] and pulmonary artery smooth muscle.[31] NIH database, GEO profiles, showed *ACTN3* was expressed in the patients with dilated cardiomyopathy. [a] Other database generated by FANTOM5 project showed *ACTN3* was expressed in adult left ventricular atrium.[b] The sarcomeric α-actinin-2 and -3 exist as a homo and heterodimers, respectively.[13] These dimers bind to Z-disc-associated alternatively spliced PDZ motif-containing protein (ZASP), myotilin, titin, and vinculin.[32] Among these proteins, deficiency of ZASP, titin, and vinculin has been reported to cause DCM.[33-35] Therefore, α-actinin-3 may play a role in cardiac function together with these proteins..

In DMD, it is anticipated that active exercise is related to increased workload for the heart resulting in worse cardiac outcomes. Endurance exercise using voluntary wheel running[36,37] and swimming[38] declined cardiac function and increased fibrosis in the *mdx* mice, which is the most common animal model for DMD. Since healthy individuals with *ACTN3* null genotype showed lower muscle strength,[17,18] there may be a possible interaction between ACTN3 deficient skeletal muscle and heart in DMD. In this study, we compared the age at loss of ambulation and found no significant difference among different *ACTN3* genotypes. Previously, Hougarth *et al.*[20] also examined the correlation

between the age at loss of ambulation and *ACTN3* genotypes in the patient with DMD, however, no significant difference was observed. They showed a significant effect of *ACTN3* genotype on both isolated muscle strength and overall muscle function. It was interesting that heterozygous 577RX genotype showed the slowest velocity on the 10-meter walk test and the least strength on grip, elbow extensor/flexor, and knee flexor on quantitative muscle testing. At the moment, it is difficult to conclude *ACTN3* null genotype influences poor physical activity, and *ACTN3* null genotype-related poor cardiac outcome is related to the function of ACTN3 deficient skeletal muscles in DMD.

As a result of respiratory care and glucocorticoid use, patients with DMD are living longer; therefore, treating cardiomyopathy is an important clinical priority.[39] Despite advances in the diagnosis and management of cardiac complications, cardiomyopathy remains the leading cause of death in DMD. Accurately predicting the risk of cardiomyopathy in DMD patients is essential to improve cardiac outcomes. The progression of cardiomyopathy is variable in patients with DMD, and even DMD siblings with the same mutation in the *DMD* gene show wide variability in the development of cardiomyopathy.[40]

Studies have sought to identify potential genetic modifiers to explain this variation with limited evidence described to date.[8] We have recently studied the relationship between Dp116, a non-muscle dystrophin isoform, and cardiac function and found that DMD patients with mutations in the Dp116 coding region showed a higher cardiac dysfunction (LVEF<53%)-free survival rate,[9] suggesting that

lack of Dp116 is a protective factor for LVEF reduction. In this study, we also examined the impact of Dp116 and *ACTN3* genotype on cardiomyopathy; however, no meaningful findings were observed (data not shown). These results indicate that *ACTN3* genotype independently relates to the progression of cardiomyopathy in DMD.

Given the association between *ACTN3* 577XX null genotype and early progression of DCM, it is important to consider the cardiac management of these patients. One patient in our study with the *ACTN3* null genotype was excluded from survival rate analyses due to a very early onset of DCM. The patient's first echocardiogram performed at 8 years of age showed a reduced EF of 48.3%, and despite optimal treatment, the LV dilation reached 55 mm at the age of 11 years. Taken together with the results of our survival analysis, this observation suggests that patients with the *ACTN3* null genotype are at high risk for the development of DCM, and should receive more frequent cardiac assessment than currently recommended. To prevent early onset of DCM, we propose the implementation of a novel cardiac assessment protocol (Figure 4).

Fig. 4

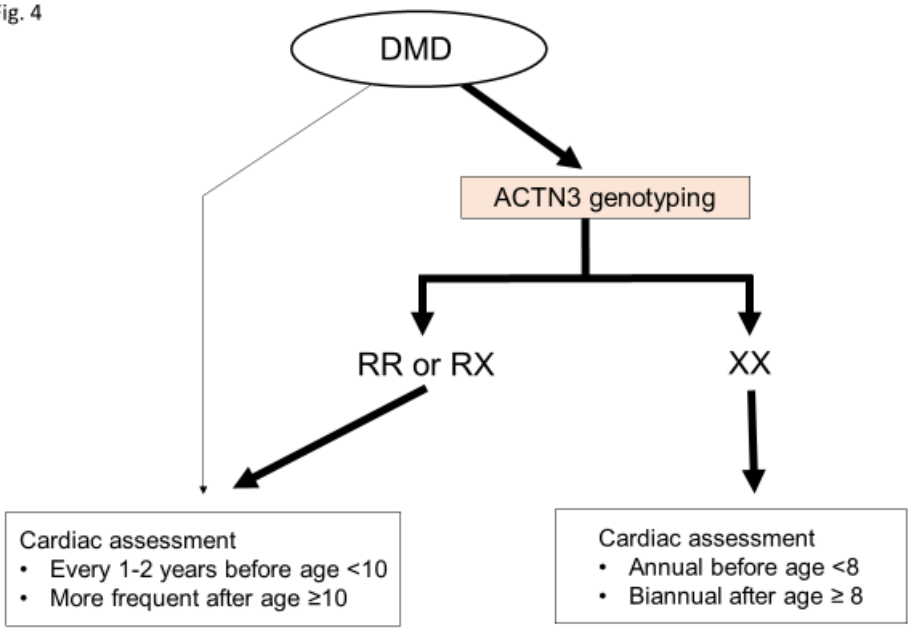

Figure 4. Recommended cardiac assessment based on the *ACTN3* genotype. For patients with XX genotype, annual cardiac assessment is recommended until the age of 8 years, and biannually thereafter. Patients with RR or RX genotype should follow current cardiac assessment guidelines

First, patients with DMD should undergo genomic analysis to identify *ACTN3* genotype at the time of initial diagnosis. Those with XX genotype should undergo annual cardiac assessment until the age of 8 years and biannually thereafter given the increased risk for DCM. Those with RR or RX genotype should be managed according to current cardiac assessment guidelines. We believe this novel cardiac assessment model based on *ACTN3* genotype could improve clinical care and cardiac outcomes in DMD patients.

This study has some limitations. First, this is a retrospective observational study conducted in a single center with all the inherent limitations of such a study e.g. referral filter bias. Second, this study was limited by the small size of the DMD cohort. Further large-scale studies are warranted to examine applicability of this study's results; unfortunately, this may not be possible given the rarity of this neuromuscular disorder.

This study revealed the correlation between *ACTN3* genotype and DCM in DMD. *ACTN3* null XX genotype enhances the progression of DCM in patients with DMD. To improve cardiac outcomes, *ACTN3* genotype should be determined at the time of diagnosis of DMD.

Acknowledgments

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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 of interest.

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Table 1. Clinical characteristics of *ACTN3* genotypes

	RR	RX	XX	P
Participants	13	44	20	
Initial echocardiographic examination age	8.8	9.5	9.2	0.69
(Median, inter-quartile range, years)	(7.4 – 12.2)	(7.9 – 12.4)	(7.9-11.5)	
Final echocardiographic examination age	14.7±3.8	15.6±5.1	13.6±4.2	0.28
(Mean±SD, years)				
Oral prednisolone use (%)	7 (53.9)	14 (31.8)	8 (40.0)	0.34
ACE inhibitor use (%)	6 (46.2)	19 (43.2)	11 (55.0)	0.77
Beta-blocker use (%)	6 (46.2)	16(34.0)	9 (45.0)	0.59
Median age at loss of ambulation (years)	10	11	9	0.19*

RR, Patient with wild type c.1729C (p.577R) variant in two alleles; RX, Patient with a single nucleotide variant of C.1729C>T (p. R577X); XX, Patient with a single nucleotide variant in both alleles. *Compared by log-rank test.

Table 2. Clinical characteristics of *ACTN3* positive and null genotypes

	ACTN3 positive genotype	*ACTN3* null genotype	P
Participants	57	20	
Initial echocardiographic examination age	9.3	9.15	0.66
(Median, inter-quartile range, years)	(7.8 - 12.2)	(7.9 – 11.5)	
Final echocardiographic examination age	15.4±4.8	13.6±4.2	0.14
(Mean±SD, years)			
Oral prednisolone use (%)	21 (36.8)	8 (40.0)	0.80
ACE inhibitor use (%)	25 (43.9)	11 (55.0)	0.44
Beta-blocker use (%)	22 (38.6)	9 (45.0)	0.79
Median age at loss of ambulation (years)	11	9	0.21*

*Compared by log-rank test

Supplementary Table 1

List of identified mutations the *DMD* gene and polymorphisms in the *ACTN3* gene.

KUCG Number	*DMD* mutation	Predicted effect of *DMD* mutation	*ACTN3* polymorphism
30	c.6283C>T	nonsense mutation in exon 7	RX
145	c.8218-?_8390+?dup	duplication of exon 56	RX
147	c.6423C>A	nonsense mutation in exon 44	RX
170	c.7661-?_8027+?del	deletion of exons 53 to 54	RX
181	c.6615-?_7098+?del	deletion of exons 46 to 48	XX
201	c.531-?_4071+?del	deletion of exons 7 to 29	RX
202	c.6615-?_7542+?del	deletion of exons 46 to 51	XX
213	c.6291-?_6438+?del	deletion of exon 44	XX
214	c.6439-?_7309+?del	deletion of exons 45 to 50	RX
225	c.5899C>T	nonsense mutation in exon 41	XX
245	c.3959delC	1bp deletion in exon 29	RX
258	c.6439-?_7660+?del	deletion of exons 45 to 52	RX
263	c.6291-?_6438+?del	deletion of exon 44	RX
277	c.1773delA	1bp deletion in exon 15	RX
294	c.2168+1G>C	splicing mutation at exon 17	RX
327	c.6615-?_7912+?del	deletion of exons 46 to 47	XX
342	c.7654delG	1bp deletion in exon 52	RR
348	c.6615-?_7200+?del	deletion of exons 46 to 49	RX
376	c.2169-?_5326+?del	deletion of exons 18 to 37	RX
377	c.1062G>A	nonsense mutation in exon 10	RX
382	c.94-?_2169+?del	deletion of exons 3 to 17	RX
394	c.7661-?_8027+?del	deletion of exons 53 to 54	RR
395	c.7543-?_7660+?del	deletion of exon 52	RR
399	c.6615-?_7098+?del	deletion of exons 46 to 48	RX
427	c.6615-?_7098+?del	deletion of exons 46 to 48	XX
434	c.3347_3350delAGAA	4bp deletion in exon 25	XX
435	c.7310-?_7542+?del	deletion of exon 51	RR
436	c.5561delT	1bp deletion in exon 39	XX

441	c.10498_10499delAG	2bp deletion in exon 74	RX
447	c.8218-?_9224+?dup	duplication of exons 56 to 62	RX
449	c.961-?_1602+?del	deletion of exons 10 to 13	RR
456	c.7817G>A	nonsense mutation in exon 53	RX
472	c.2804-?_6438+?dup	duplication of exons 22 to 44	RX
474	c.6913-?_7309+?del	deletion of exons 48 to 50	RX
478	c.9913G>T	nonsense mutation in exon 68	RX
501	c.5899C>T	nonsense mutation in exon 41	XX
505	c.4729delC	1bp deletion in exon 34	XX
512	c.6613dupA	1bp insertion in exon 45	RR
559	c.6805C>T	nonsense mutation in exon 47	RX
571	c.355C>T	nonsense mutation in exon 5	XX
577	c.5551C>T	nonsense mutation in exon 39	RX
581	c.6615-?_7542+?del	deletion of exons 46 to 51	RX
603	c.7310-?_9084+?dup	duplication of exons 51 to 60	RX
610	c.6439-?_7309+?del	deletion of exons 48 to 50	RX
623	c.6439-?_6614+?del	deletion of exon 45	RX
630	c.6291-?_6912+?del	deletion of exons 44 to 47	RX
643	c.8460G>A	nonsense mutation in exon 57	RX
651	c.5899C>T	nonsense mutation in exon 41	RX
656	c.6913-?_7660+?del	deletion of exons 48 to 52	RX
664	c.650-?_1602+?del	deletion of exons 8 to 13	XX
681	c.7099-?_7660+?del	deletion of exons 49 to 52	RX
689	c.650-?_3276+?del	deletion of exons 8 to 24	RX
708	c.94-?_649+?del	duplication of exons 10 to 11	RX
712	c.1329_1331+5delCAAGTAAG	splicing mutation at exon 11	RR
726	c.783dupT	1bp insertion in exon 8	XX
740	c.961-?_1331+?dup	duplication of exons 10 to 11	RR
763	c.6291-?_6438+?del	deletion of exon 44	RX
767	c.650-?_1992+?dup	duplication of exons 8 to 16	XX
791	c.7543-?_7660+?del	deletion of exon 52	XX
795	c.4536_4540delGAGTG	5bp deletion in exon 33	XX
809	c.2677C>T	nonsense mutation in exon21	RX
810	c.650-?_2292+?del	deletion of exons 8 to 18	RX
815	c.961-?_2169+?del	deletion of exons 10 to 17	XX
818	c.3908_3909delCT	2bp deletion in exon 28	RX

847	c.2419C>T	nonsense mutation in exon20	RX
851	c.650-?_2292+?del	deletion of exons 8 to 18	RR
857	c.9807+2714C>T	deep intronic mutation in intron 67	RR
865	c.6615-?_6912+?del	deletion of exons 46 to 47	RR
877	c.4414C>T	nonsense mutation in exon32	RX
879	c.7657C>T	nonsense mutation in exon52	RX
898	c.10108 C>T	nonsense mutation in exon70	RR
907	c.724C>T	nonsense mutation in exon8	RX
915	c.6615-?_7872+?del	deletion of exons 46 to 53	RR
921	c.9851G>A	nonsense mutation in exon68	RX
923	c.7780C>T	nonsense mutation in exon53	XX
926	c.6439-?_6614+?del	deletion of exon 45	XX
939	c.6291-?_6438+?del	deletion of exon 44	XX

KUCG; Kobe University Clinical Genetics

Highlights

- *ACTN3* genotype is a genetic modifier for Duchene muscular dystrophy
- *ACTN3* genotype with risk of dilated cardiomyopathy in patients was studied
- *ACTN3* 577XX null genotype has low left ventricular dilation-free survival rate
- *ACTN3* 577XX null genotype is a risk factor for left ventricular dilation