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Serum Cardiac Troponin I is an Early Biomarker for Cardiomyopathy in Duchenne and Becker Muscular Dystrophies

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Ethical Public Statement

We confirm that we have read the Journal's position on issues involved in ethical

publication and affirm that this report is consistent with those guidelines.

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ABSTRACT

Introduction/Aims: We described transition of cardiac troponin I (cTnI) by ages in Duchenne and Becker muscular dystrophy (DMD and BMD). We then compared cTnI levels in both groups and assessed whether cTnI is a biomarker of cardiac dysfunction. Furthermore, we evaluated the contribution of the *ACTN3* genotype to the serum levels of cTnI as XX null genotype is reported to facilitate cardiac dysfunction in DMD patients.

Methods: Serum cTnI values obtained from 127 DMD and 47 BMD patients were retrospectively analyzed. The relationship between cTnI and echocardiography data or *ACTN3* XX genotype was assessed.

Results: Both cTnI levels and the proportion of abnormal cTnI levels were significantly higher in DMD patients than BMD patients, especially in the second decade of life. In DMD, cTnI level reached a maximum at 13 years, and LVEF became abnormal at a year after the maximum level. In BMD, cTnI level peaked at the age of 14 years, and LVEF became abnormal at the three years after the peak. Decreased left ventricular ejection fraction was observed after cTnI elevation in both populations. cTnI levels by age in DMD patients with *ACTN3* XX genotype tended to increase highly and early.

Discussion: Myocardial injury indicated by cTnI elevation was more common and

severe in DMD patients. cTnI elevation preceding cardiac dysfunction may represent an early phase of cardiomyopathy progression and may be a biomarker for early detection of cardiomyopathy in DMD and BMD patients. The *ACTN3* XX genotype may be a risk factor for early myocardial injury.

Keywords: *ACTN3*; cardiomyopathy; dystrophinopathy; heart failure; muscular dystrophy

INTRODUCTION

Recently, improved respiratory management has contributed to better respiratory status in patients with Duchenne and Becker muscular dystrophy (DMD and BMD), and end-stage heart failure (HF) following cardiomyopathy is the main cause of death.[1]However, early detection of cardiac dysfunction is difficult, especially in DMD, because of the early loss of ambulation, which reduces the load on the heart, thereby reducing cardiac symptoms.[2,3] Early diagnosis of cardiac involvement is important because timely initiation of cardioprotective medications relieves cardiac dysfunction and delays heart muscle remodeling.[4,5] Although echocardiography is a standard modality, it is not always adequate for detecting the early, clinically asymptomatic phase of cardiac dysfunction because of body habitus or scoliosis.[6] Cardiovascular magnetic resonance imaging (cMRI) is useful for detecting early cardiac dysfunction.[7] However, it is costly, not globally available, and requires sedation in young patients.[3,8] Despite the increased awareness of cardiac dysfunction in those patients, an average delay of 2.5 years between the onset of symptoms and the diagnosis of HF has been reported.[9] Therefore, accurate and low-cost biomarkers for early detection of cardiomyopathy are needed.

Cardiac troponin I (cTnI) is a member of the troponin complex and a major component of myofibrils.[10] cTnI is uniquely expressed in cardiac muscles,[11] and

because it appears in the blood following cardiac injury, serum cTnI is used as a specific cardiac injury marker for acute myocardial infarction (AMI) in adults.[12] Little is known about its characteristics and diagnostic value of serum cTnI in DMD and BMD patients.[11]

The *ACTN3* gene encodes alpha-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), of *ACTN3* that results in the replacement of arginine (R) with a premature stop codon (X) at amino acid 577, leading to the deficiency of alpha-actinin-3 in the individuals with the XX genotype.[14] We have previously reported that the *ACTN3* XX genotype is associated with a lower left ventricular (LV) dilation-free survival rate in DMD,[15] suggesting that *ACTN3* is a genetic modifier of cardiomyopathy.

The first aim of this study was to describe an overall picture of the distribution of serum cTnI by ages in DMD and BMD patients. The second aim was to compare cTnI levels between patients with DMD or BMD. The third aim was to assess the progress of cTnI level and cardiac function changes and determine whether cTnI is a biomarker for early detection of cardiac dysfunction. Furthermore, we evaluated the contribution of the *ACTN3* genotype to the serum levels of cTnI.

METHODS

Study design and subjects

This retrospective, clinical observational study was conducted with the approval of the Ethics Committee of Kobe University (Approval No. 1534). Informed consent was obtained from the patients or their parents.

We reviewed the electronic charts of patients with DMD or BMD at Kobe University Hospital. Between August 1, 1991 and May 15, 2019, 459 DMD and 104 BMD patients were followed up with. Of these, patients whose cTnI was measured during the regular checkup were enrolled in this study. Blood sampling in each individual was performed during the regular checkup, not during unscheduled visits. Therefore, samples were not obtained when symptoms suspected of ischemic heart disease, including myocardial infarction, such as chest pain or dyspnea, were present. The diagnoses of DMD or BMD were confirmed by the identification of mutations in the *DMD* gene and/or immunohistochemistry in skeletal muscle (detailed background is shown in Table S1). Gene mutations were analyzed in both genomic DNA and mRNA extracted from muscle or lymphocytes, as described previously.[16]

First, we studied the distribution of cTnI in patients with 127 DMD and 47 BMD patients by age. Second, we compared serum cTnI levels and proportions of abnormal

cTnI levels between those patients. To compare serum cTnI values and the proportions of patients with abnormal cTnI values, the highest serum cTnI value for each patient (only one value per patient over a lifetime) was adopted for statistical analysis. Thus, 127 and 47 values were collected for patients with DMD and BMD, respectively. Then, we studied changes in serum cTnI levels and left ventricular ejection fraction (LVEF) in the second decade of life in those patients because abnormal cTnI values were generally found in the second decade of life in patients with DMD and BMD[17,18]. Then, as we hypothesize that the elevation of serum cTnI is an initial abnormal finding suggesting cardiac involvement, annual changes in serum cTnI levels and echocardiographic findings were described. Finally, we studied *ACTN3* genotype and serum cTnI levels in patients with DMD. We have previously reported that the *ACTN3* XX genotype is related to the early onset of dilated cardiomyopathy in DMD patients.[15] To evaluate the contribution of the *ACTN3* genotype to cTnI level, patients with DMD were grouped into three genotypes: the RR, RX, and XX genotypes. As the *ACTN3* genotype was analyzed only for patients whose genomes were conserved, the genotype was determined in 73 of the 127 DMD patients. Genotypes RR, RX, and XX were identified in 15 (20.55 %), 37 (50.68 %), and 21 (28.77%) of the 73 patients, respectively (detailed background is shown in Table S1).

Serum cTnI measurements and reference values

Serum cTnI levels were measured with Architect STAT cardiac troponin I assay (Abbott Diagnostics) until April 14, 2015. Thereafter, the assay method was changed to Architect STAT highly sensitive TnI assay (Abbott Diagnostics) using the Architect *i*2000$_{SR}$ platform in our hospital. Between the two assays, there were no significant differences in the cTnI values were reported, with a correlation coefficient of 0.98.[19] The most significant difference between the two assays was the limit of detection. In our hospital, when the value of cTnI measured by the former assay, the lowest detection value was 0.03 and defined the values as zero if the value was less than 0.03 (the values were 0.02 or 0.01). When the value of cTnI was measured by the latter assay, the lowest detection value was 0.01 and defined the values as zero if the value was less than 0.01. The upper limit was at least 50 ng/mL and 50 ng/mL, respectively, and the coefficient of variation of the 99th percentile values of healthy individuals was 10-12% (28 pg/mL) and 4% (26 pg/mL), respectively, as reported by the manufacturer in the package inserts. According to previous literature and the manufacturer's recommendation, abnormal serum cTnI levels for both assays were defined as follows: ≥0.07 ng/mL

(patient's age: 1< years ≤10), ≥0.05 ng/mL (patient's age: 10< years ≤18), and ≥0.03 ng/mL (patient's age: >18 years).[20,21]

Echocardiography

A detailed echocardiogram method has been previously described.[16] All echocardiograms were performed by the same author (TY). As a regular checkup, an echocardiographic evaluation of patients with DMD or BMD was generally scheduled annually until the age of 12 years and biannually thereafter. All patients were placed in the supine position during the investigation. Cardiac dysfunction was defined as left ventricular ejection fraction (LVEF) <53%.[16] Echocardiogram data was obtained on the same day as serum cTnI sampling.

General medication use

As for the general medication use in our hospital, when a DMD patient turns 5 years old, we tell the family about the advantages, such as extend the period until you become unable to walk, possible slowing the progression of cardiac disease, and disadvantages of glucocorticoids use, such as side effects including obesity, osteoporosis, etc. and ask them to choose whether to start taking glucocorticoids or not. On the other hand,

basically, steroids are not used for BMD patients. As for the use of cardiac medications, we start beta-blocker and ACE-I if dyskinetic wall motion is found by echocardiography for DMD and BMD patients. Treatment is not started or stopped depending on the value of cTnI.

ACTN3 genotyping

The *ACTN3* genotype was determined only in DMD patients whose genomic DNA was conserved in our laboratory. Genomic DNA was isolated using standard phenol-chloroform extraction methods. *ACTN3* exon 15 was amplified by polymerase chain reaction (PCR) as previously described.[15] The purified PCR-amplified products were sequenced using the Premix sequencing system (Fasmac Co., Ltd., Kanagawa, Japan). If patients had a variant in two alleles of c.1729C (p.577R), the *ACTN3* genotype was defined as RR. If patients had a single nucleotide variant of c.1729C>T (p. R577X) in one allele, the *ACTN3* genotype was defined as RX, and in two alleles, as XX.

Statistical analysis

Data were expressed as numbers and percentages or medians and interquartile ranges (IQR) or mean and standard deviation (SD). Mann-Whitney's U test or Fisher's exact

test was used, as appropriate, for statistical analysis of the results. Linear approximation and coefficient of determination (R^2) and were calculated by EXCEL2019 (Microsfoft ®) . A difference was considered statistically significant when the p-value was <0.05. Analyses were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). To describe an overall picture of the distribution of cTnI by ages in DMD and BMD patients, all cTnI data obtained from enrolled DMD and BMD patients were used. To compare cTnI levels between DMD and BMD patients, the highest serum cTnI value for each patient over a lifetime was adopted for statistical analysis. To assess the progress of cTnI level and cardiac function changes in patients with DMD or BMD by ages, multiple measurements of cTnI or LVEF per person with DMD or BMD were included. When an examination was performed more than two times in a year of age, the earliest one was used for assessment.

RESULTS

Distribution of cTnI in patients with DMD and BMD by age

During the first decade of life, serum cTnI levels were rarely elevated, and abnormal values were generally found in the second decade in both groups (Fig. 1A and 1B). The cTnI levels stabilized at a low level after the third decade in both groups. Detailed

transition of cTnI measured in each patient by age was shown in Fig. S1(supplement).

Comparison of serum cTnI levels and proportions of abnormal cTnI levels between DMD patients and BMD patients

The background data of those patients at the time of serum cTnI assay are displayed in Table 1. There was no statistical difference in age between those groups. Angiotensin-converting enzyme inhibitors, beta-blockers, steroids, non-invasive ventilation were more commonly used in DMD patients. The comparison of the highest value of serum cTnI for each patient between DMD and BMD groups is shown in Table 2. The median serum cTnI level in the DMD group was 0.06 ng/mL, which was higher than that in the BMD group (0.01 ng/mL). The median serum cTnI (1< years ≤10, 10< years ≤18) was statistically higher in patients with DMD than in those with BMD. In contrast, in patients >18 years, there was no statistical difference. The proportion of patients with abnormal serum cTnI levels was statistically larger in patients with DMD within the age range of 10< years ≤18 than in BMD patients.

Changes in serum cTnI levels and LVEF in the second decade of life

Annual changes in serum cTnI levels and echocardiographic findings were described

(Fig. 2). In DMD patients, the median serum cTnI level was elevated to the abnormal range (cTnI ≥0.05 ng/mL) at 11 years of age. It reached a maximum at 13 years, and thereafter, it decreased with age and returned to the normal range at 16 years of age (Fig. 2A). Median LVEF decreased with age and became abnormal (LVEF <53%) at the age of 14 years—a year after the maximum cTnI level was observed. Cardiac dysfunction (LVEF <53%) was observed three years after abnormal median serum cTnI levels (Fig. 2C). In BMD patients, serum cTnI value also increased with age and peaked at the age of 14 years. Thereafter, it decreased with age (Fig. 2B). In contrast to the finding in DMD patients, the median peak of serum cTnI (0.04 ng/mL) in BMD patients did not exceed the upper limit of the standard value (≥0.05 ng/mL). LVEF decreased with age and became abnormal at the age of 17 years (Fig. 2D)—three years after the age at which cTnI peaked in BMD patients. In the present study, only 15 DMD patients had abnormal cTnI measured when LVEF was normal, followed by abnormal LVEF. In these patients, the median age (IQR) at which cTnI first showed abnormal values was 11 (10,13) and the median age at which maximum cTnI was 13 (12,13), and the median age at which LVEF showed abnormal values for the first time was 14 (13,15). These results are same shown in Fig. 2A and 2C. Mean difference between age at which LVEF showed abnormal and age at which cTnI first showed abnormal values was 2.5 (1.0)

[year (SD)]. Mean difference between age at which LVEF showed abnormal values for the first time and age at which maximum cTnI was 1.4 (1.3) [year (SD)]. The linear approximation and R^2 of those results in 15 patients were shown in Fig. 3A and 3B. Both analyses were well fit for the linear approximation [R^2=0.79 (A) and R^2=0.59 (B)]. Furthermore, we performed Bland–Altman analysis and it shows the accuracy of agreement between those factors (Fig. 3C and 3D). As for BMD, only one BMD patient had abnormal cTnI measured when LVEF was normal, followed by abnormal LVEF. Thus, we could not analyze that in BMD patients. Detailed transition of cTnI measured in each patient by age was shown in Fig. 4.

ACTN3 genotype and serum cTnI levels in patients with DMD

Patients with the A*CTN3* XX genotype had maximum cTnI levels at the age of 11 years. On the other hand, in patients with RR and RX genotypes, cTnI peaked at the ages of 14 and 12 years, respectively (Fig. 5A). The highest median cTnI values were 0.16, 0.21, and 0.29 ng/mL in the RR, RX, and XX groups, respectively. Patients with the XX genotype had a higher cTnI peak value at an earlier stage of disease than those with other genotypes. To determine the impact of ACTN3 deficiency, patients were divided into two groups—XX group and RR and RX group—and we compared the annual

change in cTnI levels between the two groups. The peak of cTnI in the XX group occurred three years earlier than in the RR and RX group (Fig. 5B).

DISCUSSION

We confirmed that higher cTnI levels were generally found in the second decade of life in patients with DMD and BMD. Second, the median serum cTnI level by age was higher in DMD patients until the age of 18 years, and abnormal cTnI values were more common in the DMD group than in the BMD group. In addition, the median maximum cTnI levels were found one year before the median abnormal LVEF value in DMD patients and three years before in BMD patients. Finally, we found that the *ACTN3* XX genotype showed higher cTnI elevation earlier than the other two genotypes in DMD patients.

cTnI is a member of the troponin complex and a major component of myofibrils, and it is uniquely expressed in cardiac muscles.[22] It appears in the blood following cardiac injury and is a specific cardiac injury marker.[7] Its levels are elevated not only in acute but also in chronic pathogenic conditions. Recently, increasing cTnI levels have been shown in patients with cardiomyopathy or chronic HF in the general population.[23,24] In the present study, 55.9% and 31.9% of the patients with DMD and

BMD, respectively, had elevated cTnI levels. Although Kan et al. reported that DMD patients who had acute cardiomyopathy with acute chest pain had elevated cTnI levels and diffuse ST changes on ECG,[25] none of the patients in our study complained of symptoms related to acute myocardial injury such as chest pain or dyspnea. Moreover, echocardiography and ECG did not show any sign of AMI. These results, therefore, indicated that chronic myocardial injury caused elevation of cTnI levels in patients with DMD and BMD in our study.

In the present study, we found higher serum cTnI levels with increasing age in DMD patients compared with BMD patients, and the proportion of patients with abnormal serum cTnI levels was larger in the DMD group at all ages and in the age range of 10 <years ≤18 when compared with the BMD group. The onset of symptomatic cardiomyopathy occurs in the mid-teen years to 20s in DMD patients[26,27] and 30s to 40s in BMD patients.[28] These results indicate that DMD generally has an earlier and more severe cardiac phenotype than BMD. The difference in the level of cTnI between the two groups of patients is considered to reflect the different degrees of myocardial damage in the two muscular disorders.

Previous studies have reported the utility of cTnI for evaluating cardiac function, especially in patients with DMD. Matsumura et al. reported that most DMD patients

showed higher levels of cTnI in the second decade of their lives; however, no obvious correlation between cTnI and LVEF or brain natriuretic peptide was observed.[29] Hammere-Lercher et al. reported that all patients with DMD, with a mean age of 7.5 years, had cTnI levels below the upper reference limit (URL), and there was no relation of cTnI level to clinical evidence of cardiac failure.[11] Castro-Cago et al. also reported no relationship between cTnI levels and cardiac function.[30] These reports suggest that cTnI cannot be used to evaluate cardiac function. However, as shown in our study, the cTnI level was transiently elevated in the second decade before the decline of LVEF. This indicated that the cTnI level was not associated with cardiac function at the time of measurement, but later, it was.

The most significant limitation of our study is that individual calculations were not made of the lag between cTnI and LVEF abnormalities. There were 103 DMD patients whose cTnI and echocardiographic results were measured simultaneously. Interesting to note, 4 patients aged 19 years or older had normal cTnI and normal LVEF, but no patients who had abnormal cTnI showed normal LVEF after the age (Fig. 4). These may suggest that DMD patients who did not show abnormal cTnI during second year of life showed normal LVEF. However, it was difficult to say so because the short follow-up period for each patient. On the contrary, 10 patients had normal cTnI and abnormal

LVEF after 16 years. However, it is unknown whether these patients exhibited abnormal cTnI levels before age 15 or remained normal. Further research of individual calculations made of the lag between cTnI and LVEF abnormalities will be needed.

Recently, myocardial fibrosis (MF) in DMD patients has been demonstrated using cMRI with late gadolinium enhancement (LGE), which revealed that subepicardial fibrosis was the main characteristic of DMD patients.[31] When the myocardium is injured, damaged cardiomyocytes are repaired by recruitment, proliferation, and activation of cardiac fibroblasts, which produce extracellular matrix components, resulting in the formation of fibrotic scars.[32] Remarkably, MF has been reported in DMD cardiomyopathy before the onset of myocardial dysfunction in young patients with DMD.[33] We hypothesize that serum cTnI levels may increase with the progression of MF because the observed timings of fibrosis and cTnI rise are the same (early second decade of life). Recently, Sonia et al. reported that cTnI values correlated with cMRI findings in patients with DMD cardiomyopathy.[34] They showed that cTnI levels in DMD patients with mild LGE were significantly increased compared to those in patients without LGE. These studies and the present study indicate that cTnI, a standard marker for AMI, may have the potential to become an alternative, cost-effective, and noninvasive biomarker for detecting early signs of cardiac injury. In fact, cTnI has

gained popularity as a biomarker in the diagnosis of HF,[35] and the cost of cTnI assay will be estimated to be 10–100 times less than that of cardiac imaging such as echocardiography and cMRI.[8]

As measurable plasma cTnI is found in the healthy population,[18,36] the abnormal value of cTnI is recommended to exceed the 99th percentile URL.[37] However, there is no internationally accepted standard for the 99th percentile URL of cTnI, although a wide range of variables has been used as the 99th percentile URL.[38] Caselli et al. recently reported plasma cTnI levels in healthy neonates, children, and adolescents; 357 participants had a high sensitive immunoassay similar to that used in our study. In their study, the cTnI showed the highest value in the first weeks of life, and it decreased progressively up to adulthood. Therefore, the 99th percentile URL needs to be defined according to age. They reported that the 99th percentile URL was age-dependent; it was 61.3 ng/L for the whole population minus neonates and infants (1< years ≤18) and 41.3 ng/L for the group of adolescents (10< years ≤18) [20]. Unfortunately, they did not report the 99th percentile URL for toddlers (1< years ≤10). Therefore, we decided to define the 99th percentile URL according to the patient's age as follows: ≥0.07 ng/mL, 1< years ≤10; ≥0.05 ng/mL, 10< years ≤18; and ≥0.03 ng/mL, >18 years (manufacturer's recommendation).

In the present study, we also examined the relationship between the *ACTN3* genotype and cTnI levels in patients with DMD and found that the maximum cTnI level in patients with *ACTN3* XX genotype was observed a few years earlier compared with the other two genotypes. These results suggest that patients with the XX genotype may have a higher risk for myocardial injury. Interestingly, the existence of alpha-actinin-3 has been reported not only in skeletal muscles but also in human fetal and adult hearts.[39] We recently reported that the XX genotype is related to a lower LV dilation-free survival rate in patients with DMD.[15] The impact of alpha-actinin-3 deficiency on cardiomyopathy progression was not elucidated in this study. Our results, however, indicate that alpha-actinin-3-deficient myocardium can be sensitive to mechanical and/or hypoxic damage that induces elevation of cTnI levels.

This study has some limitations. First, it was a retrospective observational study and was subject to selection bias. Second, although we evaluated a relatively large number of patients with DMD and BMD compared to previous studies, the number of participants may not be enough to allow generalization of our results to larger cohorts. However, the rarity of these muscular disorders may make it difficult to conduct studies on larger samples. Third, no patient had cTnI level measured over a long follow-up period; therefore, we could not elucidate precise changes in serum cTnI level for each

patient by age. Furthermore, our study failed to reveal if early medications, such as steroid or cardiac medicines can suppress the release of cTnI or prevent deterioration of cardiac function or not because treatment is not started or stopped depending on the value of cTnI in the present study. Fourth, two different assays were used in the present study (Architect STAT cardiac troponin I assay until April 14, 2015. Thereafter, Architect STAT highly sensitive TnI assay), although there were no significant differences in the cTnI values between the two assays. Finally, we only examined the relationship between the *ACTN3* genotype and cTnI levels in patients with DMD and not BMD because the DNA samples preserved in BMD patients were not enough to be analyzed (only 14 MD patients of *ACTN3* could be analyzed, and we failed during same study period of DMD (from 6-16 year-old). Our findings may positively impact cardiac care by supporting the use of cTnI as a biomarker for cardiomyopathy.

In conclusion, we evaluated and compared serum cTnI levels and cardiac function in patients with DMD or BMD in a large cohort. cTnI elevation preceding cardiac dysfunction may represent an early phase of cardiomyopathy progression and may be a biomarker for early detection of cardiomyopathy in those patients. The *ACTN3* XX genotype may be a risk factor for early myocardial injury. We consider this genotype should be used as a stratification criteria in terms of DMD cardiomyopathy research or

clinical trial studies. Thus, we would like to recommend to check *ACTN3* genotype before these researches.

Author contributions

H.Y.: Conceptualization, Methodology, Formal Analysis, Investigation, Writing—Original Draft. **H.A.:** Conceptualization, Methodology, Investigation, Writing—Review & Editing, Supervision, Project Administration, Funding Acquisition. **T.Y.:** Resources, Writing—Review & Editing, Supervision. **M.M.:** Writing—Review & Editing, Supervision. **K.I.:** Writing—Review & Editing, Supervision.

Abbreviations: ACTN3: alpha-actinin-3; AMI: acute myocardial infarction; BMD: Becker muscular dystrophy; cMRI: cardiovascular magnetic resonance imaging; cTnI: cardiac troponin I; DMD: Duchenne muscular dystrophy; ECG: electrocardiogram; HF: heart failure; IQR: interquartile range; LGE: late gadolinium enhancement; LV: left ventricular; LVEF: left ventricular ejection fraction; MF: myocardial fibrosis; PCR: polymerase chain reaction; URL: upper reference limit

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Table 1: Patients' background characteristics

	DMD (n=127)	BMD (n=47)	P-value
Age, median (IQR), years	13 (8, 18)	15 (10, 18)	0.19
Medication, n (%)			
ACEI	42 (33.1)	7 (14.9)	0.022[$]
ARB	3 (2.4)	0 (0)	0.56
beta-blocker	42 (33.1)	5 (10.6)	0.004[$]
diuretic	3 (2.4)	0 (0)	0.56
steroid	23 (18.1)	0 (0)	0.0006[$]
Motor, n (%)			
gait	42 (33.1)	46 (97.9)	<0.0001[$]
walker	1 (0.8)	0 (0)	1.0000
wheelchair	84 (66.1)	1 (2.1)	<0.0001[$]
Respiratory management, n (%)			
non-invasive ventilation	13 (10.2)	0 (0)	0.021[$]
ventilator	0 (0)	0 (0)	-

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; IQR, interquartile range

[$]Values are statistically significant according to Fisher's exact test.

Table 2: Comparison of serum cTnI levels and proportion of abnormal cTnI levels in DMD and BMD patients

	DMD (n=127)	BMD (n=47)	P-value
cTnI, median (IQR), ng/mL			
Total	0.06 (0.00, 0.16)	0.01 (0.00, 0.07)	0.031*
(1< years ≤10)	0.00 (0.00, 0.04)	0.00 (0.00, 0.00)	0.048*
(10< years ≤18)	0.13 (0.07, 0.49)	0.04 (0.01, 0.15)	0.039*
(18< years)	0.03 (0.00, 0.08)	0.02 (0.01, 0.04)	0.74
Abnormal cTnI levels, n/n (%)			
Total	71/127 (55.9%)	15/47 (31.9%)	0.006$
(1< years ≤10)	8/44 (18.2%)	1/14 (7.1%)	0.43
(10< years ≤18)	49/57 (86.0%)	10/22 (45.5%)	0.0005$
(18< years)	14/26 (53.8%)	4/11 (36.4%)	0.48

Abbreviations: BMD, Becker muscular dystrophy; cTnI, cardiac troponin I; DMD, Duchenne muscular dystrophy; IQR, interquartile range

*Values are statistically significant according to Mann-Whitney's U test.

$Values are statistically significant according to Fisher's exact test.

Figure legends:

Figure 1. Serum cTnI levels in patients with DMD and BMD by age

A total of 174 patients (127 DMD and 47 BMD patients) were enrolled. In all, 529 and 131 serum cTnI values were obtained from 127 DMD (A) and 47 BMD (B) patients, respectively. All data on serum cTnI levels, including multiple measurements for each person by age. The vertical axis shows the serum cTnI concentration (ng/mL) logarithmically. Dotted lines in A and B show upper limits of reference values. Markedly high values (>2.0 ng/mL) were observed in both patient groups during the second decade (Fig. 1A and B); these values were obtained from three DMD and two BMD patients. The three DMD patients who showed markedly high values had deletions of exons 56–62, small insertions in exon 8 (c.783dupT), and small deletion in exon 18 (c.2230_2231delAG). The mutations in the two BMD patients were small deletions in exon 27 (c.3613delG)[28] and deep intron mutation in intron 4 (c.265-463A>G). There was no specific predisposition to any mutation position or type. None of the patients showed symptoms such as chest pain or electrocardiogram (ECG) findings related to AMI at the time of cTnI measurement. cTnI, cardiac troponin I; BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy

Figure 2. Serum cTnI levels and LVEF in the second decade of life in patients with DMD and BMD

Dotted lines in A and B show upper limits of reference values. Dotted lines in C and D, indicate the assessment of cardiac dysfunction (LVEF <53%). A box and whisker plot show the first quartile to the third quartile. A horizontal line goes through the box at the median. The upper and lower whiskers represent scores outside the middle 50%.

cTnI, cardiac troponin I; BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; LVEF, left ventricular ejection fraction

Figure 3. The Linear approximation and assessment of the agreement of the age at which cTnI first showed abnormal values and the age at which LVEF showed abnormal values for the first time and the age at which maximum cTnI and the median age at which LVEF showed abnormal values for the first time.

The Linear approximation of the age at which LVEF showed abnormal values for the first time (Age A) and the age at which cTnI first showed abnormal values (Age B) was shown in Fig. 3A. The Linear approximation of Age A and the age at which maximum cTnI (Age C) was shown in Fig. 3B. For the assessment of the agreement, Bland–

Altman plots were shown in Fig. 3C and 3D. The middle blue line represents the mean difference between Age A and Age B (2.5 years) (Fig. 3C) and Age A and Age C (1.4 years)(Fig. 3D). The upper and lower dotted lines shows the 95% limits of agreement (±1.96 standard deviation [SD]) fall within 0.5 to 4.4 years (Fig. 3C) and –1.1 to 3.9 years (Fig. 3D).

Figure 4. The transition of cTnI measured in each patient whose cTnI and echocardiographic results were measured simultaneously by age.

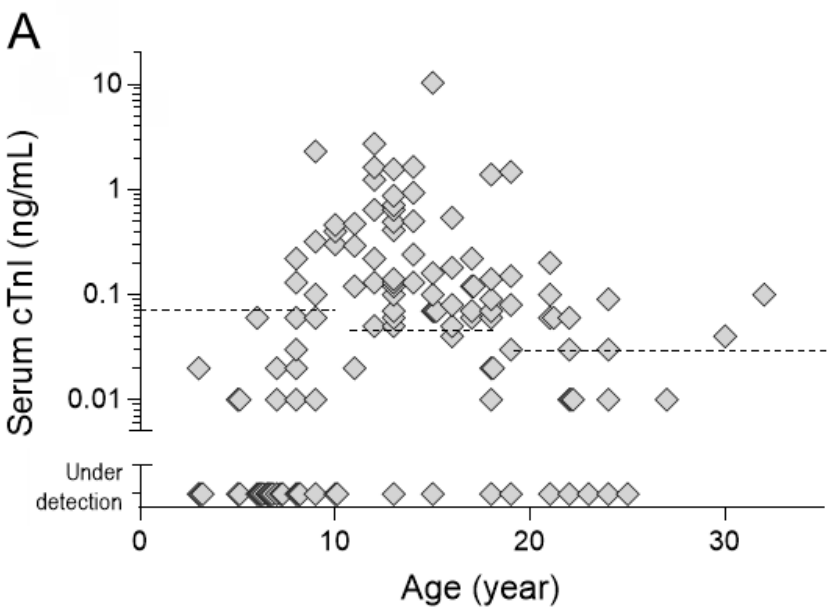
There were 103 DMD patients whose cTnI and echocardiographic results were measured simultaneously. Among these patients, those who showed abnormal values in cTnI during the course are indicated by red dots and red lines, and those who did not show abnormal values in cTnI in the course are indicated by blue dots and blue lines. In the course, 6 patients showed cTnI was normal and normal LVEF between the ages of 10 and 18 years, which is the key age. In addition, 4 patients aged 19 years or older had normal cTnI and normal LVEF, but no patients who had abnormal cTnI showed normal LVEF after the age. In addition, 10 patients had normal cTnI and abnormal LVEF after 16 years. However, it is unknown whether these patients exhibited abnormal cTnI levels before age 15 or remained normal.

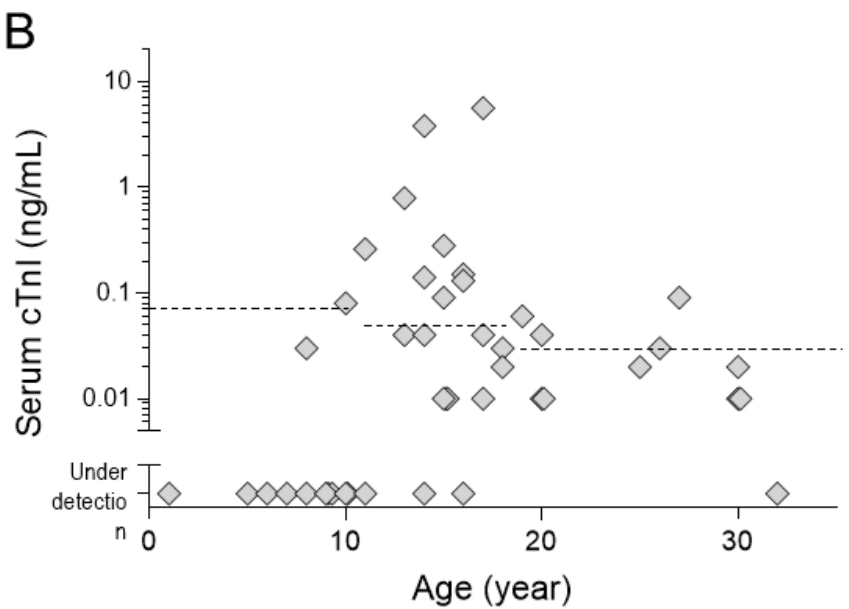
Figure 5. Changes in serum cTnI levels in patients with different *ACTN3* genotypes

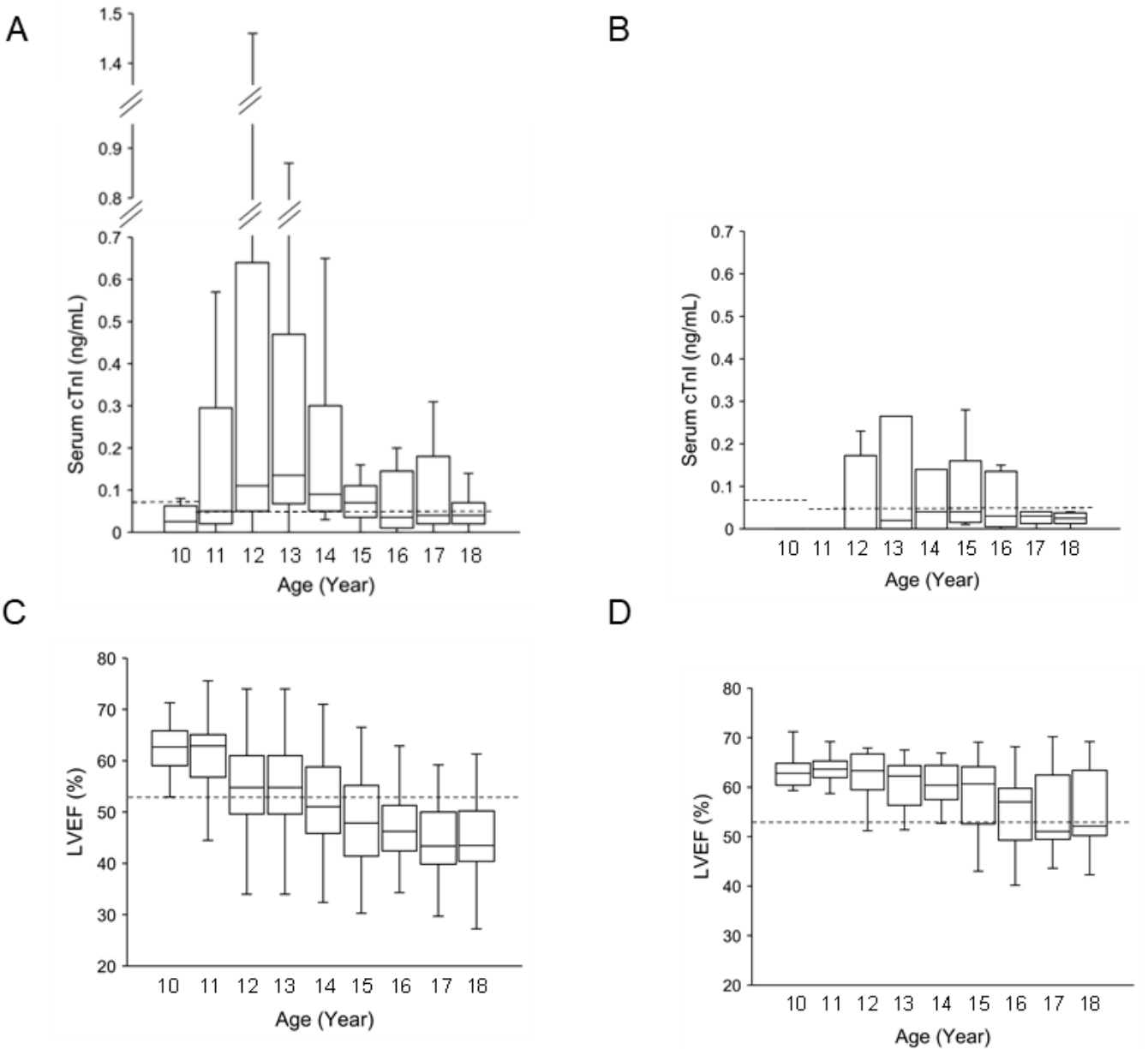
The XX genotype showed an early onset of cTnI elevation (a). When compared between the XX genotype group and the RR and RX genotype groups, serum cTnI in the XX group peaked three years earlier (b). Data are represented as median, the positive vertical bar represents 75th percentile, and the negative vertical bar represents 25th percentile. RR, RX, and XX represent the *ACTN3* 577RR, 577RX, and 577XX genotypes, respectively. cTnI, cardiac troponin I

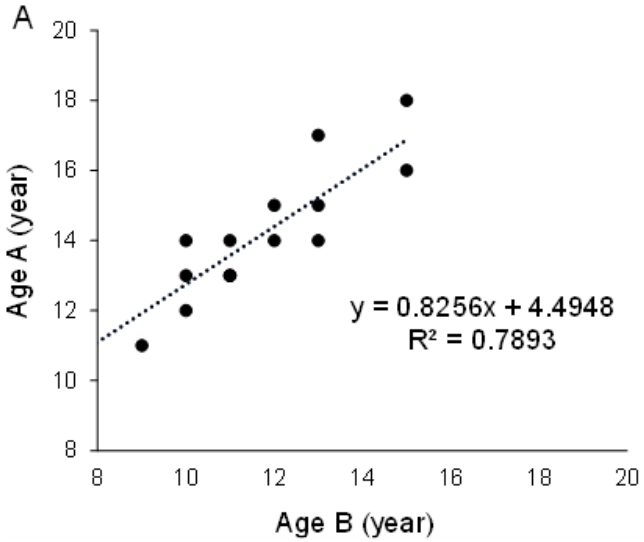
Figure S1. The transition of cTnI measured in each patient by age.

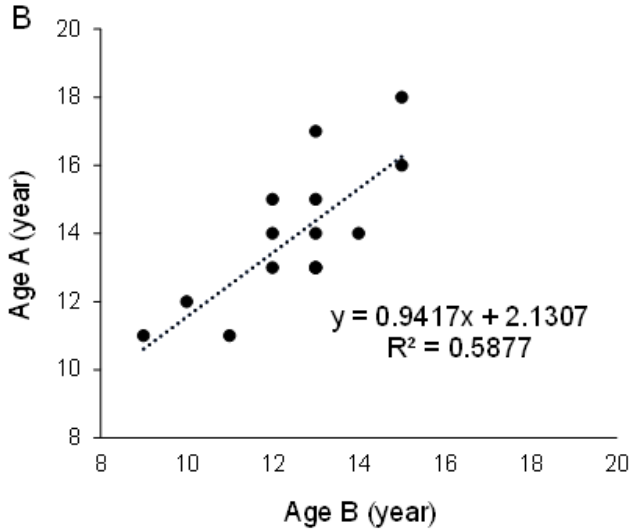
Five hundred twenty nine and one hundred thirty one serum cTnI values obtained from 127 DMD patients in A and 47 BMD patients in B, respectively.

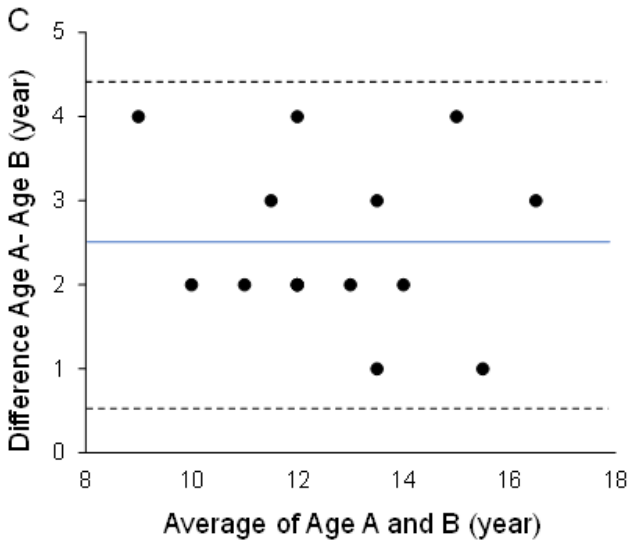

A
Serum cTnI (ng/mL)
10
1
0.1
0.01
Under detection
0
10
20
30
Age (year)

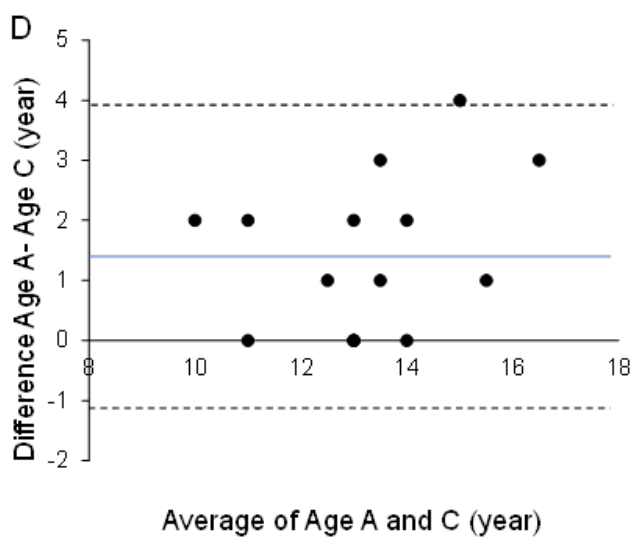

B
Serum cTnI (ng/mL)
10
1
0.1
0.01
Under detectio n
0
10
20
30
Age (year)

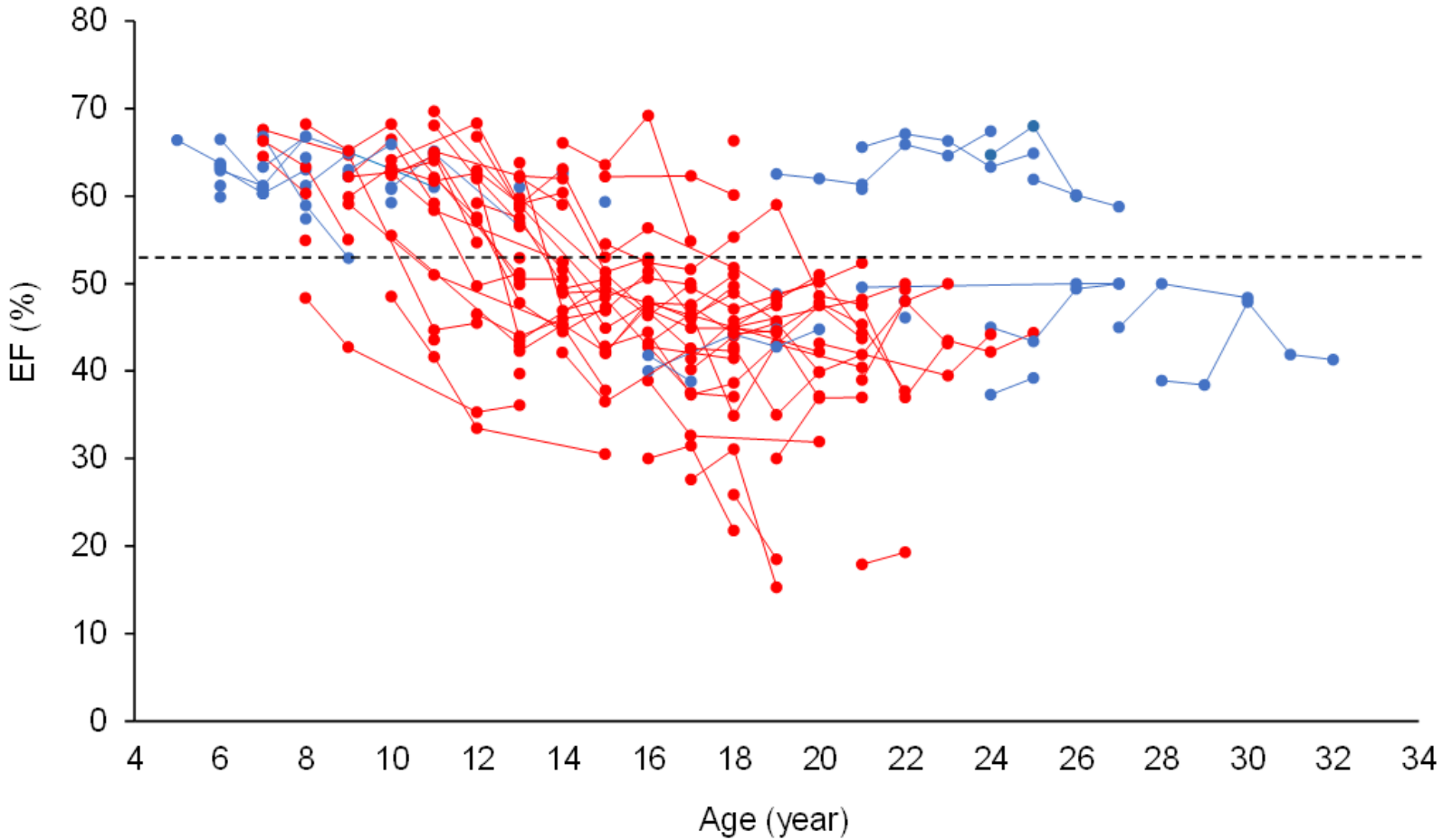

A
B
C
D
Serum cTnI (ng/mL)
LVEF (%)
Age (Year)
10 11 12 13 14 15 16 17 18
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.4 1.5
20 30 40 50 60 70 80

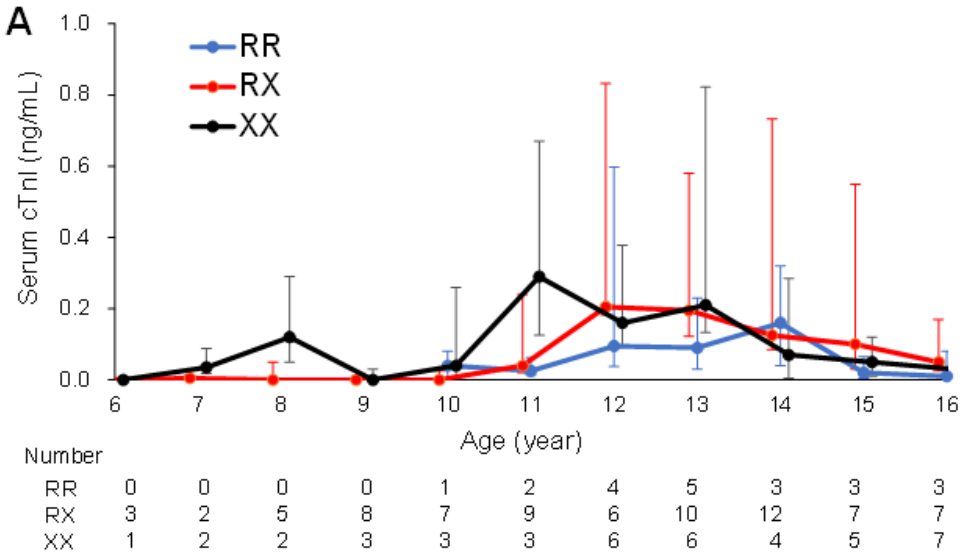

A
Age A (year)
Age B (year)
y = 0.8256x + 4.4948
R² = 0.7893

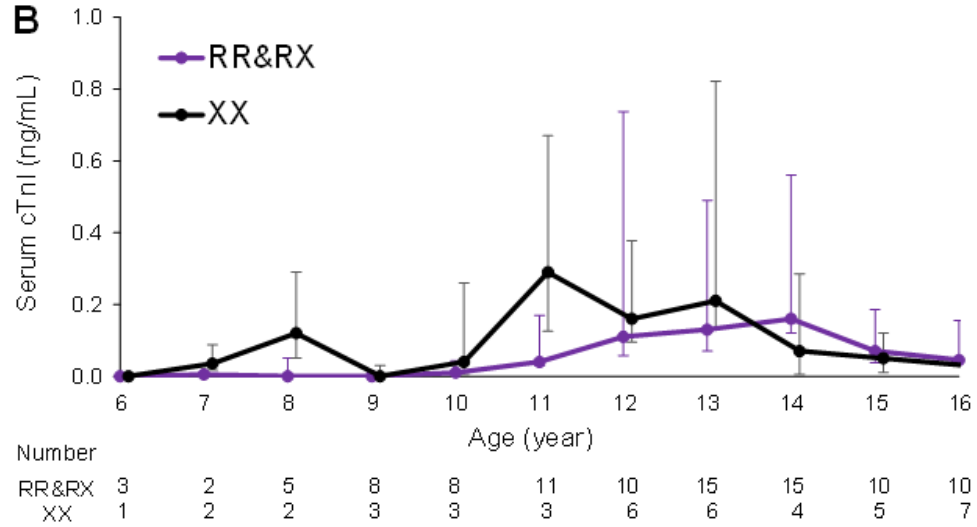

B
Age A (year)
Age B (year)
y = 0.9417x + 2.1307
R² = 0.5877


C
Difference Age A- Age B (year)
Average of Age A and B (year)


D
Difference Age A- Age C (year)
Average of Age A and C (year)


80
70
60
50
40
30
20
10
0
EF (%)
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
Age (year)


A
RR
RX
XX
Serum cTnI (ng/mL)
1.0
0.8
0.6
0.4
0.2
0.0
6 7 8 9 10 11 12 13 14 15 16
Age (year)
Number
RR 0 0 0 0 1 2 4 5 3 3 3
RX 3 2 5 8 7 9 6 10 12 7 7
XX 1 2 2 3 3 3 6 6 4 5 7


B
RR&RX
XX
Serum cTnI (ng/mL)
1.0
0.8
0.6
0.4
0.2
0.0
6 7 8 9 10 11 12 13 14 15 16
Age (year)
Number
RR&RX 3 2 5 8 8 11 10 15 15 10 10
XX 1 2 2 3 3 3 6 6 4 5 7

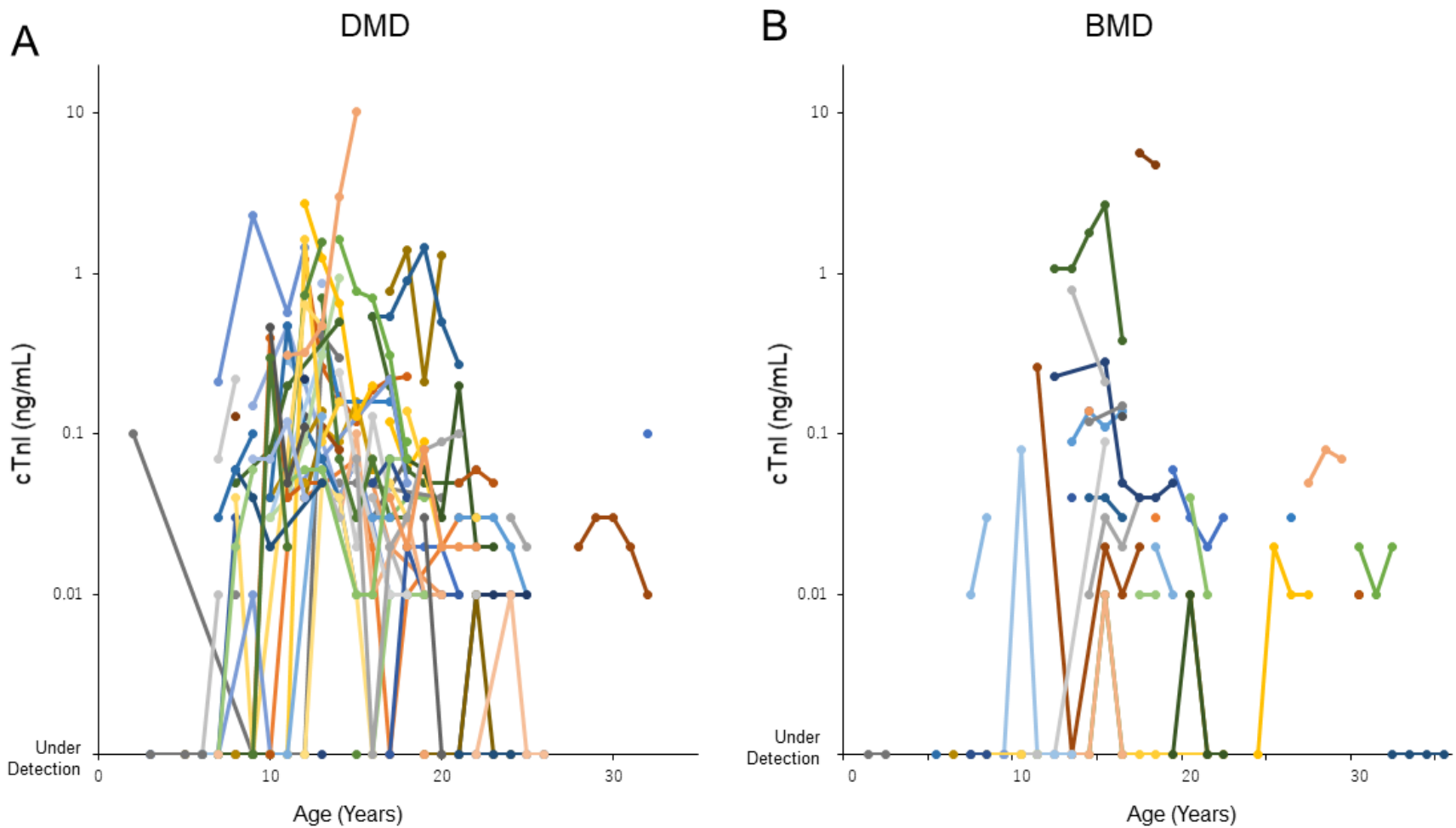

A
DMD
B
BMD
cTnI (ng/mL)
10
1
0.1
0.01
Under Detection
0
10
20
30
Age (Years)

Table S1. A list of dystrophin mutations and corresponding *ACTN3* genotypes for each patients

N.O.	KUCG number	*DMD* mutation	Predicted effect of *DMD* mutation	*ACTN3* genotype
1	5	c.580C>T	nonsense mutation in exon 7	XX
2	26	c.(6912+1_6913-1)_(7309+1_7310-1)del	deletion of exons 48 to 50	
3	27	c.(6912+1_6913-1)_(7660+1_7661-1)del	deletion of exons 48 to 52	
4	30	c.6283C>T	nonsense mutation in exon 43	RX
5	58	c.354G>A	nonsense mutation in exon 5	RX
6	99	c.(6438+1_6439-1)_(8027+1_8028-1)del	deletion of exons 45 to 54	
7	112	c.(6912+1_6913-1)_(7309+1_7310-1)del	deletion of exons 48 to 50	
8	170	c.(7660+1_7661-1)_(8027+1_8028-1)del	deletion of exons 53 to 54	RX
9	174	c.(2622+1_2623-1)_(7660+1_7661-1)del	deletion of exons 21 to 52	XX
10	181	c.(6614+1_6615-1)_(7098+1_7099-1)del	deletion of exons 46 to 48	XX
11	201	c.(530+1_531-1)_(4071+1_4072-1)del	deletion of exons 7 to 29	RX
12	202	c.(6614+1_6615-1)_(7542+1_7543-1)del	deletion of exons 46 to 51	XX
13	210	c.(7542+1_7543-1)_(7660+1_7661-1)del	deletion of exon 52	
14	213	c.(6290+1_6291-1)_(6438+1_6439-1)del	deletion of exon 44	XX
15	214	c.(6438+1_6439-1)_(7309+1_7310-1)del	deletion of exons 45 to 50	RX
16	231	c.1376_1377delAG	2bp deletion in exon 12	
17	245	c.3959delC	1bp deletion in exon 29	RX
18	251	c.(264+1_265-1)_(6290+1_6291-1)dup	duplication of exons 5 to 43	
19	264	c.2089A>T	nonsense mutatin in exon17	
20	277	c.1773delA	1bp deletion in exon 15	RX
21	294	c.2168+1G>C	splicing mutation at exon 17	RX
22	327	c.(6614+1_6615-1)_(6912+1_6913-1)del	deletion of exons 46 to 47	XX
23	336	c.(649+1_650-1)_(1331+1_1332-1)dup	duplication of exons 8 to 11	
24	342	c.7654delG	1bp deletion in exon 52	RR
25	343	c.(6614+1_6615-1)_(7200+1_7201-1)dup	duplication of exons 46 to 49	
26	348	c.(6614+1_6615-1)_(7200+1_7201-1)del	deletion of exons 46 to 49	RX
27	376	c.(2168+1_2169-1)_(5325+1_5326-1)del	deletion of exons 18 to 37	RX
28	394	c.(7660+1_7661-1)_(8027+1_8028-1)del	deletion of exons 53 to 54	RR
29	395	c.(7542+1_7543-1)_(7660+1_7661-1)del	deletion of exon 52	RR
30	411	c.(649+1_650-1)_(2949+1_2950-1)del	deletion of exons 8 to 22	RR
31	415	c.(7309+1_7310-1)_(8027+1_8028-1)del	deletion of exons 51 to 54	
32	426	c.(6438+1_6439-1)_(8390+1_8391-1)del	deletion of exons 45 to 56	
33	434	c.3347_3350delAGAA	4bp deletion in exon 25	XX
34	435	c.(7309+1_7310-1)_(7542+1_7543-1)del	deletion of exon 51	RR
35	441	c.10498_10499delAG	2bp deletion in exon 74	RX
36	442	c.(6438+1_6439-1)_(7309+1_7310-1)del	deletion of exons 45 to 50	XX
37	444	c.(264+1_265-1)_(649+1_650-1)del	deletion of exons 5 to 7	RX
38	447	c.(8217+1_8218-1)_(9224+1_9225-1)dup	duplication of exons 56 to 62	RX
39	449	c.(960+1_961-1)_(1602+1_1603-1)del	deletion of exons 10 to 13	RR
40	472	c.(2803+1_2804-1)_(6438+1_6439-1)dup	duplication of exons 22 to 44	RX
41	480	c.(2803+1_2804-1)_(6438+1_6439-1)dup	duplication of exons 22 to 44	RX
42	501	c.5899C>T	nonsense mutation in exon 41	XX
43	505	c.4729delC	1bp deletion in exon34	XX
44	516	c.(6438+1_6439-1)_(8027+1_8028-1)del	deletion of exons 45 to 54	RX
45	559	c.6805C>T	nonsense mutation in exon 47	RX
46	581	c.(6614+1_6615-1)_(7542+1_7543-1)del	deletion of exons 46 to 51	RX
47	588	c.(6912+1_6913-1)_(8027+1_8028-1)del	deletion of exons 48 to 54	RR
48	608	c.(7309+1_7310-1)_(7872+1_7873-1)del	deletion of exons 51 to 53	XX
49	614	c.(7542+1_7543-1)_(7660+1_7661-1)del	deletion of exon 52	XX
50	615	c.(6614+1_6615-1)_(6912+1_6913-1)del	deletion of exons 46 to 47	
51	643	c.8460G>A	nonsense mutation in exon 57	RX
52	644	c.(6912+1_6913-1)_(7309+1_7310-1)del	deletion of exons 48 to 50	
53	681	c.(7098+1_7099-1)_(7660+1_7661-1)del	deletion of exons 49 to 52	RX
54	682	c.2622+1G>A	splicing mutation at exon 20	XX
55	689	c.(649+1_650-1)_(3277-96_3336)del	deletion of exons 8 to 24 and part of the 5' side of exon 25	RX
56	700	c.7255G>T	nonsense mutation in exon 50	RX
57	708	c.(93+1_94-1)_(649+1_650-1)dup	duplication of exons 3 to 7	RX
58	712	c.1329_1331+5delCAAGTAAG	splicing mutation at exon 11	RR
59	726	c.783dupT	1bp insertion in exon 8	XX
60	728	c.1627delA	1bp deletion in exon 14	XX
61	733	c.(6614+1_6615-1)_(7200+1_7201-1)del	deletion of exons 46 to 49	RX
62	740	c.(960+1_961-1)_(1331+1_1332-1)dup	duplication of exons 10 to 11	RR
63	763	c.(6290+1_6291-1)_(6438+1_6439-1)del	deletion of exon 44	RX
64	765	c.(7309+1_7310-1)_(7542+1_7543-1)del	deletion of exon 51	RR
65	792	c.(7660+1_7661-1)_(9084+1_9085-1)del	deletion of exons 53 to 60	XX
66	806	c.(6438+1_6439-1)_(9286+1_9287-1)del	deletion of exons 45 to 63	
67	810	c.(649+1_650-1)_(2292+1_2293-1)del	deletion of exons 8 to 18	RX
68	813	c.(8217+1_8218-1)_(9224+1_9225-1)del	deletion of exons 56 to 62	
69	818	c.3908_3909delCT	2bp deletion in exon 28	RX
70	847	c.2419C>T	nonsense mutation in exon 20	RX
71	851	c.(649+1_650-1)_(2292+1_2293-1)del	deletion of exons 8 to 18	RR
72	853	c.(7200+1_7201-1)_(7309+1_7310-1)del	deletion of exon 50	RX
73	857	c.9807+2714C>T	deep intronic mutation in intron 67	RR
74	870	c.(6912+1_6913-1)_(7309+1_7310-1)del	deletion of exons 48 to 50	
75	883	c.(93+1_94-1)_(2292+1_2293-1)del	deletion of exons 3 to 18	XX
76	885	c.(9084+1_9085-1)_(9807+1_9808-1)del	deletion of exons 61 to 67	
77	899	c.9361+1G>A	splicing mutation at exon 64	RR
78	907	c.724C>T	nonsense mutation in exon 8	RX
79	915	c.(6614+1_6615-1)_(7872+1_7873-1)del	deletion of exons 46 to 53	RR
80	921	c.9851G>A	nonsense mutation in exon 68	RX
81	922	c.(649+1_650-1)_(5922+1_5923-1)del	deletion of exons 8 to 41	RX
82	923	c.7780C>T	nonsense mutation in exon 53	XX
83	924	c.(6438+1_6439-1)_(7660+1_7661-1)del	deletion of exons 45 to 52	
84	926	c.(6438+1_6439-1)_(6614+1_6615-1)del	deletion of exon 45	XX
85	938	c.(7200+1_7201-1)_(8027+1_8028-1)dup	duplication of exons 50 to 54	RX
86	939	c.(6290+1_6291-1)_(6438+1_6439-1)del	deletion of exon 44	XX
87	946	c.(1149+1_1150-1)_(1331+1_1332-1)dup	duplication of exon 11	
88	954	c.(6614+1_6615-1)_(7872+1_7873-1)del	deletion of exons 46 to 53	
89	963	c.(6614+1_6615-1)_(7098+1_7099-1)del	deletion of exons 46 to 48	
90	966	c.(?_-244)_(8937+1_8938-1)del	deletion of exons 1 to 59	RX
91	981	c.(6912+1_6913-1)_(10553+1_10554-1)dup	duplication of exons 48 to 74	
92	982	c.(6912+1_6913-1)_(10553+1_10554-1)dup	duplication of exons 48 to 74	
93	995	c.2230_2231delAG	2bp deletion in exon 18	RX
94	1012	c.8914C>T	nonsense mutation in exon 59	RX
95	1014	c.(6614+1_6615-1)_(6912+1_6913-1)del	deletion of exons 46 to 47	
96	1016	c.(7098+1_7099-1)_(7660+1_7661-1)del	deletion of exons 49 to 52	
97	1017	c.9351delG	1bp deletion in exon 64	XX
98	1019	c.(2168+1_2169-1)_(6438+1_6439-1)del	deletion of exons 18 to 44	
99	1033	c.(960+1_961-1)_(1331+1_1332-1)del	deletion of exons 10 to 11	
100	1034	c.(960+1_961-1)_(1331+1_1332-1)del	deletion of exons 10 to 11	
101	1037	c.(649+1_650-1)_(1992+1_1993-1)dup	duplication of exons 8 to 16	
102	1039	c.8608C>T	nonsense mutation in exon 58	
103	1041	c.(6438+1_6439-1)_(7660+1_7661-1)del	deletion of exons 45 to 52	
104	1060	c.1590delA	1bp deletion in exon 13	
105	1067	c.(6912+1_6913-1)_(7660+1_7661-1)del	deletion of exons 48 to 52	
106	1083	c.(6438+1_6439-1)_(6614+1_6615-1)del	deletion of exon 45	
107	1089	c.(93+1_94-1)_(2292+1_2293-1)del	deletion of exons 3 to 18	
108	1090	c.(6912+1_6913-1)_(7660+1_7661-1)del	deletion of exons 48 to 52	
109	1092	c.2302C>T	nonsense mutaiton in exon 19	
110	1100	c.(649+1_650-1)_(9084+1_9085-1)del	deletion of exons 8 to 60	
111	1109	c.4375C>T	nonsense mutation in exon 32	
112	1127	c.(7098+1_7099-1)_(7309+1_7310-1)dup	duplication of exons 49 to 50	
113	1132	c.(1602+1_1603-1)_(2168+1_2169-1)del	deletion of exons 14 to 17	
114	1137	c.(6438+1_6439-1)_(7660+1_7661-1)del	deletion of exons 45 to 52	RR
115	1141	c.(6762+1_6763-1)_(8027+1_8028-1)del	deletion of exons 47 to 54	
116	1142	c.(6438+1_6439-1)_(6614+1_6615-1)del	deletion of exon 45	
117	1147	c.(7200+1_7201-1)_(7309+1_7310-1)del	deletion of exon 50	
118	1150	c.(7309+1_7310-1)_(7542+1_7543-1)del	deletion of exon 51	RX
119	1151	c.(7309+1_7310-1)_(7542+1_7543-1)del	deletion of exon 51	
120	1160	c.(7872+1_7873-1)_(8027+1_8028-1)del	deletion of exon 54	
121	1169	c.(2803+1_2804-1)_(3432+1_3433-1)dup	duplication of exons 22 to 25	
122	1211	c.7678C>T	nonsense mutation in exon 53	
123	1216	c.8608C>T	nonsense mutation in exon 58	
124	1218	c.2650C>T	nonsense mutation in exon 21	
125	1219	c.(7309+1_7310-1)_(7872+1_7873-1)del	deletion of exons 51 to 53	
126	1246	c.(264+1_265-1)_(2168+1_2169-1)del	deletion of exons 5 to 17	
127	1272	c.3151C>T	nonsense mutaiton in exon 23	

N.O.	KUCG number	*DMD* mutation	Predicted effect of *DMD* mutation	*ACTN3* genotype
1	154	c.6117G>A	splicing muation at exon 42	XX
2	194	c.3276+2T>G	splicing muation at exon 24	XX
3	284	c.(6912+1_6913-1)_(7542+1_7543-1)del	deletion of exons 48 to 51	
4	290	c.9225-285A>G	deep intron mutation in intron 62	RX
5	293	c.3432+1G>A	splicing muation at exon 25	RX
6	338	r.[10224_10553del]	splicing erros without any mutation in gDNA	RR
7	357	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
8	358	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
9	414	c.5434_5437delTTCA	4bp deletion in exon 38	RX
10	486	c.(6438+1_6439-1)_(7872+1_7873-1)del	deletion of exons 45 to 53	
11	487	c.(6438+1_6439-1)_(7872+1_7873-1)del	deletion of exons 45 to 53	
12	579	c.3613delG	1bp deletion in exon 27	XX
13	580	c.(93+1_94-1)_(649+1_650-1)del	deletion of exons 3 to 7	RX
14	616	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	RR
15	735	c.(6438+1_6439-1)_(7098+1_7099-1)del	deletion of exons 45 to 48	RX
16	750	c.(6438+1_6439-1)_(7098+1_7099-1)del	deletion of exons 45 to 48	
17	751	c.(31+1_32-1)_(649+1_650-1)del	deletion of exons 2 to 7	XX
18	752	c.(31+1_32-1)_(649+1_650-1)del	deletion of exons 2 to 7	XX
19	776	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
20	797	c.4303G>T	nonsense mutation in exon 31	
21	807	c.(31+1_32-1)_(649+1_650-1)del	deletion of exons 2 to 7	
22	845	c.(10223+1_10224-1)_(10262+1_10263-1)del	deletion of exon 71	RR
23	854	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
24	889	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	
25	891	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	
26	908	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	
27	931	c.(4845+1_4846-1)_(6438+1_6439-1)del	deletion of exons 35 to 44	
28	969	not confrimed yet		
29	1011	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	XX
30	1048	c.(2292+1_2293-1)_(5922+1_5923-1)del	deletion of exons 19 to 41	
31	1099	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
32	1112	c.265-463A>G	deep intron mutation in intron 4	
33	1138	c.2622G>C	splicing mutation at exon 20	
34	1139	c.93+5590T>A	deep intron mutation in intron 2	
35	1140	c.(960+1_961-1)_(4071+1_4072-1)	deletion of exons 10 to 29	
36	1161	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
37	1167	c.(960+1_961-1)_(2949+1_2950-1)del	deletion of exons 10 to 22	
38	1181	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
39	1196	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
40	1198	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
41	1203	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
42	1206	c.(960+1_961-1)_(1482+1_1483-1)del	deletion of exons 10 to 12	
43	1207	c.(6438+1_6439-1)_(7542+1_7543-1)del	deletion of exons 45 to 51	
44	1213	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
45	1238	c.(31+1_32-1)_(93+1_94-1)del	deletion of exon 2	
46	1242	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	
47	1262	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	

Abbreviations: BMD, Becker muscular dystrophy; DMD, Duchenne musucular dystrophy, KUCG, Kobe University Clinical Genetics.

Table S2. A list of dystrophin mutations and corresponding *ACTN3* genotypes for each patients

	individual					BMD				
	KUCG number	DNA change of Dystrophin gene	*DMD* mutation	Predicted effect of *DMD* mutation	*ACTN3* genotype	KUCG number	DNA change of Dystrophin gene	*DMD* mutation	Predicted effect of *DMD* mutation	*ACTN3* genotype
1	5	c.580C>T	c.580C>T	nonsense mutation in exon 7	XX	154	c.6117G>A	c.6117G>A	splicing muation at exon 42	XX
2	26	EX48_EX50del	c.(6912+1_6913-1)_(7309+1_7310-1)del	deletion of exons 48 to 50		194	c.3276+2T>G	c.3276+2T>G	splicing muation at exon 24	XX
3	27	EX48_EX52del	c.(6912+1_6913-1)_(7660+1_7661-1)del	deletion of exons 48 to 52		284	EX48_EX51del	c.(6912+1_6913-1)_(7542+1_7543-1)del	deletion of exons 48 to 51	
4	30	c.6283C>T (EX43)	c.6283C>T	nonsense mutation in exon 43	RX	290	c.9225-285A>G	c.9225-285A>G	deep intron mutation in intron 62	RX
5	58	c.354G>A (EX5)	c.354G>A	nonsense mutation in exon 5	RX	293	c.3432+1G>A	c.3432+1G>A	splicing muation at exon 25	RX
6	99	EX45_EX54del	c.(6438+1_6439-1)_(8027+1_8028-1)del	deletion of exons 45 to 54		338	EX71_EX74del	r.[10224_10553del]	splicing erros without any mutation in gDNA	RR
7	112	EX48-EX50del	c.(6912+1_6913-1)_(7309+1_7310-1)del	deletion of exons 48 to 50		357	EX45-EX47 del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
8	170	EX53_EX54del	c.(7660+1_7661-1)_(8027+1_8028-1)del	deletion of exons 53 to 54	RX	358	EX45_EX47del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
9	174	EX21_EX52del	c.(2622+1_2623-1)_(7660+1_7661-1)del	deletion of exons 21 to 52	XX	414	c.5434_5437delTTCA	c.5434_5437delTTCA	4bp deletion in exon 38	RX
10	181	EX46_EX48del	c.(6614+1_6615-1)_(7098+1_7099-1)del	deletion of exons 46 to 48	XX	486	EX45-EX53 del	c.(6438+1_6439-1)_(7872+1_7873-1)del	deletion of exons 45 to 53	
11	201	EX07_EX29del	c.(530+1_531-1)_(4071+1_4072-1)del	deletion of exons 7 to 29	RX	487	EX45_EX53del	c.(6438+1_6439-1)_(7872+1_7873-1)del	deletion of exons 45 to 53	
12	202	EX46_EX51del	c.(6614+1_6615-1)_(7542+1_7543-1)del	deletion of exons 46 to 51	XX	579	c.3613delG	c.3613delG	1bp deletion in exon 27	XX
13	210	EX52del	c.(7542+1_7543-1)_(7660+1_7661-1)del	deletion of exon 52		580	EX03_EX07del	c.(93+1_94-1)_(649+1_650-1)del	deletion of exons 3 to 7	RX
14	213	EX44del	c.(6290+1_6291-1)_(6438+1_6439-1)del	deletion of exon 44	XX	616	EX48del	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	RR
15	214	EX45_EX50del	c.(6438+1_6439-1)_(7309+1_7310-1)del	deletion of exons 45 to 50	RX	735	EX45_EX48del	c.(6438+1_6439-1)_(7098+1_7099-1)del	deletion of exons 45 to 48	RX
16	231	c.1376_1377delAG	c.1376_1377delAG	2bp deletion in exon 12		750	EX45_EX48del	c.(6438+1_6439-1)_(7098+1_7099-1)del	deletion of exons 45 to 48	
17	245	c.3959delC	c.3959delC	1bp deletion in exon 29	RX	751	EX02_EX07del	c.(31+1_32-1)_(649+1_650-1)del	deletion of exons 2 to 7	XX
18	251	EX05_EX43dup	c.(264+1_265-1)_(6290+1_6291-1)dup	duplication of exons 5 to 43		752	EX02-EX07 del	c.(31+1_32-1)_(649+1_650-1)del	deletion of exons 2 to 7	XX
19	264	c.2089A>T	c.2089A>T	nonsense mutatin in exon17		776	EX45_EX47del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
20	277	c.1773delA	c.1773delA	1bp deletion in exon 15	RX	797	c.4303G>T	c.4303G>T	nonsense mutation in exon 31	
21	294	c.2168+1G>C	c.2168+1G>C	splicing mutation at exon 17	RX	807	EX02_EX07del	c.(31+1_32-1)_(649+1_650-1)del	deletion of exons 2 to 7	
22	327	EX46_EX47del	c.(6614+1_6615-1)_(6912+1_6913-1)del	deletion of exons 46 to 47	XX	845	EX71del	c.(10223+1_10224-1)_(10262+1_10263-1)del	deletion of exon 71	RR
23	336	EX08_EX11dup	c.(649+1_650-1)_(1331+1_1332-1)dup	duplication of exons 8 to 11		854	EX45_EX47del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
24	342	c.7654delG	c.7654delG	1bp deletion in exon 52	RR	889	EX48del	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	
25	343	EX46_EX49dup	c.(6614+1_6615-1)_(7200+1_7201-1)dup	duplication of exons 46 to 49		891	EX48del	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	
26	348	EX46_EX49del	c.(6614+1_6615-1)_(7200+1_7201-1)del	deletion of exons 46 to 49	RX	908	EX48del	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	
27	376	EX18_EX37del	c.(2168+1_2169-1)_(5325+1_5326-1)del	deletion of exons 18 to 37	RX	931	EX35_EX44del	c.(4845+1_4846-1)_(6438+1_6439-1)del	deletion of exons 35 to 44	
28	394	EX53_EX54del	c.(7660+1_7661-1)_(8027+1_8028-1)del	deletion of exons 53 to 54	RR	969		not confirmed yet		
29	395	EX52del	c.(7542+1_7543-1)_(7660+1_7661-1)del	deletion of exon 52	RR	1011	EX48del	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	XX
30	411	EX08_EX22del	c.(649+1_650-1)_(2949+1_2950-1)del	deletion of exons 8 to 22	RR	1048	EX19_EX41del	c.(2292+1_2293-1)_(5922+1_5923-1)del	deletion of exons 19 to 41	
31	415	EX51_EX54del	c.(7309+1_7310-1)_(8027+1_8028-1)del	deletion of exons 51 to 54		1099	EX45_EX47del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
32	426	EX45_EX56del	c.(6438+1_6439-1)_(8390+1_8391-1)del	deletion of exons 45 to 56		1112	, deep intron mutation, alternative sp	c.265-463A>G	deep intron mutation in intron 4	
33	434	c.3347_3350delAGAA	c.3347_3350delAGAA	4bp deletion in exon 25	XX	1138	c.2622G>C, splice site mutation	c.2622G>C	splicing mutation at exon 20	
34	435	EX51del	c.(7309+1_7310-1)_(7542+1_7543-1)del	deletion of exon 51	RR	1139	c.93+5590T>A	c.93+5590T>A	deep intron mutation in intron 2	
35	441	c.10498_10499delAG	c.10498_10499delAG	2bp deletion in exon 74	RX	1140	EX10_EX29del (+α)	c.(960+1_961-1)_(4071+1_4072-1)	deletion of exons 10 to 29	
36	442	EX45_EX50del	c.(6438+1_6439-1)_(7309+1_7310-1)del	deletion of exons 45 to 50	XX	1161	EX45_EX47del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
37	444	EX05_EX07del	c.(264+1_265-1)_(649+1_650-1)del	deletion of exons 5 to 7	RX	1167	EX10_EX22del	c.(960+1_961-1)_(2949+1_2950-1)del	deletion of exons 10 to 22	
38	447	EX56_EX62dup	c.(8217+1_8218-1)_(9224+1_9225-1)dup	duplication of exons 56 to 62	RX	1181	EX45_EX47del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
39	449	EX10_EX13del	c.(960+1_961-1)_(1602+1_1603-1)del	deletion of exons 10 to 13	RR	1196	EX45_EX47del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
40	472	EX22_EX44dup	c.(2803+1_2804-1)_(6438+1_6439-1)dup	duplication of exons 22 to 44	RX	1198	EX45_EX47del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
41	480	EX22-EX44dup	c.(2803+1_2804-1)_(6438+1_6439-1)dup	duplication of exons 22 to 44	RX	1203	EX45_EX47del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
42	501	c.5899C>T	c.5899C>T	nonsense mutation in exon 41	XX	1206	EX10_EX12del	c.(960+1_961-1)_(1482+1_1483-1)del	deletion of exons 10 to 12	
43	505	c.4729delC	c.4729delC	1bp deletion in exon34	XX	1207	EX45_EX51del	c.(6438+1_6439-1)_(7542+1_7543-1)del	deletion of exons 45 to 51	
44	516	EX45_EX54del	c.(6438+1_6439-1)_(8027+1_8028-1)del	deletion of exons 45 to 54	RX	1213	EX45_EX47del	c.(6438+1_6439-1)_(6912+1_6913-1)del	deletion of exons 45 to 47	
45	559	c.6805C>T	c.6805C>T	nonsense mutation in exon 47	RX	1238	EX02del	c.(31+1_32-1)_(93+1_94-1)del	deletion of exon 2	
46	581	EX46_EX51del	c.(6614+1_6615-1)_(7542+1_7543-1)del	deletion of exons 46 to 51	RX	1242	EX48del	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	
47	588	EX48_EX54del	c.(6912+1_6913-1)_(8027+1_8028-1)del	deletion of exons 48 to 54	RR	1262	EX48del	c.(6912+1_6913-1)_(7098+1_7099-1)del	deletion of exon 48	
48	608	EX51_EX53del	c.(7309+1_7310-1)_(7872+1_7873-1)del	deletion of exons 51 to 53	XX					
49	614	EX52del	c.(7542+1_7543-1)_(7660+1_7661-1)del	deletion of exon 52	XX					
50	615	EX46_EX47del	c.(6614+1_6615-1)_(6912+1_6913-1)del	deletion of exons 46 to 47						
51	643	c.8460G>A	c.8460G>A	nonsense mutation in exon 57	RX					
52	644	EX48_EX50del	c.(6912+1_6913-1)_(7309+1_7310-1)del	deletion of exons 48 to 50						
53	681	EX49_EX52del	c.(7098+1_7099-1)_(7660+1_7661-1)del	deletion of exons 49 to 52	RX					
54	682	c.2622+1G>A	c.2622+1G>A	splicing mutation at exon 20	XX					
55	689	EX08_EX24del and EX25 partial deletion	c.(649+1_650-1)_(3277-96_3336)del	deletion of exons 8 to 24 and part of the 5' side of exon 25	RX					
56	700	c.7255G>T	c.7255G>T	nonsense mutation in exon 50	RX					
57	708	EX03_EX07dup	c.(93+1_94-1)_(649+1_650-1)dup	duplication of exons 3 to 7	RX					
58	712	c.1329_1331+5delCAAGTAAG	c.1329_1331+5delCAAGTAAG	splicing mutation at exon 11	RR					
59	726	c.783dupT	c.783dupT	1bp insertion in exon 8	XX					
60	728	c.1627delA	c.1627delA	1bp deletion in exon 14	XX					
61	733	EX46_EX49del	c.(6614+1_6615-1)_(7200+1_7201-1)del	deletion of exons 46 to 49	RX					
62	740	EX10_EX11dup	c.(960+1_961-1)_(1331+1_1332-1)dup	duplication of exons 10 to 11	RR					
63	763	EX44del	c.(6290+1_6291-1)_(6438+1_6439-1)del	deletion of exon 44	RX					
64	765	EX51del	c.(7309+1_7310-1)_(7542+1_7543-1)del	deletion of exon 51	RR					
65	792	EX53_EX60del	c.(7660+1_7661-1)_(9084+1_9085-1)del	deletion of exons 53 to 60	XX					
66	806	EX45_EX63del	c.(6438+1_6439-1)_(9286+1_9287-1)del	deletion of exons 45 to 63						
67	810	EX08_EX18del	c.(649+1_650-1)_(2292+1_2293-1)del	deletion of exons 8 to 18	RX					
68	813	EX56_EX62del	c.(8217+1_8218-1)_(9224+1_9225-1)del	deletion of exons 56 to 62						
69	818	c.3908_3909delCT	c.3908_3909delCT	2bp deletion in exon 28	RX					
70	847	c.2419C>T	c.2419C>T	nonsense mutation in exon 20	RX					
71	851	EX08_EX18del	c.(649+1_650-1)_(2292+1_2293-1)del	deletion of exons 8 to 18	RR					
72	853	EX50del	c.(7200+1_7201-1)_(7309+1_7310-1)del	deletion of exon 50	RX					
73	857	c.9807+2714C>T	c.9807+2714C>T	deep intronic mutation in intron 67	RR					
74	870	EX48_EX50del	c.(6912+1_6913-1)_(7309+1_7310-1)del	deletion of exons 48 to 50						
75	883	EX03_EX18del	c.(93+1_94-1)_(2292+1_2293-1)del	deletion of exons 3 to 18	XX					
76	885	EX61_EX67del	c.(9084+1_9085-1)_(9807+1_9808-1)del	deletion of exons 61 to 67						
77	899	c.9361+1G>A	c.9361+1G>A	splicing mutation at exon 64	RR					
78	907	c.724C>T	c.724C>T	nonsense mutation in exon 8	RX					
79	915	EX46_EX53del	c.(6614+1_6615-1)_(7872+1_7873-1)del	deletion of exons 46 to 53	RR					
80	921	c.9851G>A	c.9851G>A	nonsense mutation in exon 68	RX					
81	922	EX08_EX41del	c.(649+1_650-1)_(5922+1_5923-1)del	deletion of exons 8 to 41	RX					
82	923	c.7780C>T	c.7780C>T	nonsense mutation in exon 53	XX					
83	924	EX45-EX52del	c.(6438+1_6439-1)_(7660+1_7661-1)del	deletion of exons 45 to 52						
84	926	EX45del	c.(6438+1_6439-1)_(6614+1_6615-1)del	deletion of exon 45	XX					
85	938	EX50_EX54dup	c.(7200+1_7201-1)_(8027+1_8028-1)dup	duplication of exons 50 to 54	RX					
86	939	EX44del	c.(6290+1_6291-1)_(6438+1_6439-1)del	deletion of exon 44	XX					
87	946	EX11dup	c.(1149+1_1150-1)_(1331+1_1332-1)dup	duplication of exon 11						
88	954	EX46_EX53del	c.(6614+1_6615-1)_(7872+1_7873-1)del	deletion of exons 46 to 53						
89	963	EX46_EX48del	c.(6614+1_6615-1)_(7098+1_7099-1)del	deletion of exons 46 to 48						
90	966	EX01_EX59del	c.(?_-244)_(8937+1_8938-1)del	deletion of exons 1 to 59	RX					
91	981	EX48_EX74dup	c.(6912+1_6913-1)_(10553+1_10554-1)dup	duplication of exons 48 to 74						
92	982	EX48-EX74dup	c.(6912+1_6913-1)_(10553+1_10554-1)dup	duplication of exons 48 to 74						
93	995	c.2230_2231delAG	c.2230_2231delAG	2bp deletion in exon 18	RX					
94	1012	c.8914C>T (ex59)	c.8914C>T	nonsense mutation in exon 59	RX					
95	1014	EX46_EX47del	c.(6614+1_6615-1)_(6912+1_6913-1)del	deletion of exons 46 to 47						
96	1016	EX49_EX52del	c.(7098+1_7099-1)_(7660+1_7661-1)del	deletion of exons 49 to 52						
97	1017	c.9351delG	c.9351delG	1bp deletion in exon 64	XX					
98	1019	EX18_EX44del	c.(2168+1_2169-1)_(6438+1_6439-1)del	deletion of exons 18 to 44						
99	1033	EX10_EX11del	c.(960+1_961-1)_(1331+1_1332-1)del	deletion of exons 10 to 11						
100	1034	EX10-EX11del	c.(960+1_961-1)_(1331+1_1332-1)del	deletion of exons 10 to 11						
101	1037	EX08_EX16dup	c.(649+1_650-1)_(1992+1_1993-1)dup	duplication of exons 8 to 16						
102	1039	c.8608C>T	c.8608C>T	nonsense mutation in exon 58						
103	1041	EX45_EX52del	c.(6438+1_6439-1)_(7660+1_7661-1)del	deletion of exons 45 to 52						
104	1060	c.1590delA	c.1590delA	1bp deletion in exon 13						
105	1067	EX48_EX52del	c.(6912+1_6913-1)_(7660+1_7661-1)del	deletion of exons 48 to 52						
106	1083	EX45del	c.(6438+1_6439-1)_(6614+1_6615-1)del	deletion of exon 45						
107	1089	EX03_EX18del	c.(93+1_94-1)_(2292+1_2293-1)del	deletion of exons 3 to 18						
108	1090	EX48_EX52del	c.(6912+1_6913-1)_(7660+1_7661-1)del	deletion of exons 48 to 52						
109	1092	c.2302C>T	c.2302C>T	nonsense mutaiton in exon 19						
110	1100	EX08_EX60del	c.(649+1_650-1)_(9084+1_9085-1)del	deletion of exons 8 to 60						
111	1109	c.4375C>T、エクソン32ナンセンス	c.4375C>T	nonsense mutation in exon 32						
112	1127	EX49_EX50dup	c.(7098+1_7099-1)_(7309+1_7310-1)dup	duplication of exons 49 to 50						
113	1132	EX14_EX17del	c.(1602+1_1603-1)_(2168+1_2169-1)del	deletion of exons 14 to 17						
114	1137	EX45_EX52del	c.(6438+1_6439-1)_(7660+1_7661-1)del	deletion of exons 45 to 52	RR					
115	1141	EX47_EX54del	c.(6762+1_6763-1)_(8027+1_8028-1)del	deletion of exons 47 to 54						
116	1142	EX45del	c.(6438+1_6439-1)_(6614+1_6615-1)del	deletion of exon 45						
117	1147	EX50del	c.(7200+1_7201-1)_(7309+1_7310-1)del	deletion of exon 50						
118	1150	EX51del	c.(7309+1_7310-1)_(7542+1_7543-1)del	deletion of exon 51	RX					
119	1151	EX51del	c.(7309+1_7310-1)_(7542+1_7543-1)del	deletion of exon 51						
120	1160	EX54del	c.(7872+1_7873-1)_(8027+1_8028-1)del	deletion of exon 54						
121	1169	EX22_EX25dup	c.(2803+1_2804-1)_(3432+1_3433-1)dup	duplication of exons 22 to 25						
122	1211	c.7678C>T (in ex58)	c.7678C>T	nonsense mutation in exon 53						
123	1216	c.8608C>T	c.8608C>T	nonsense mutation in exon 58						
124	1218	c.2650C>T	c.2650C>T	nonsense mutation in exon 21						
125	1219	EX51_EX53del	c.(7309+1_7310-1)_(7872+1_7873-1)del	deletion of exons 51 to 53						
126	1246	EX05_EX17del	c.(264+1_265-1)_(2168+1_2169-1)del	deletion of exons 5 to 17						
127	1272	c.3151C>T	c.3151C>T	nonsense mutaiton in exon 23						

Abbreviations: BMD, Becker muscular dystrophy; DMD, Duchenne musucular dystrophy, KUCG, Kobe University Clinical Genetics.

RR	15
RX	37
XX	21
total	73

RR	3
RX	5
XX	6
total	14