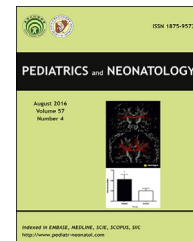


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Original Article

The natural history of the patients with Duchenne muscular dystrophy in Taiwan: A medical center experience

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Key Words

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Background: Duchenne muscular dystrophy (DMD) is the most common hereditary muscular dystrophy and caused by *DMD* gene mutation. In addition to progressive proximal muscle weakness, respiratory, orthopedic, and gastrointestinal complications are often observed in DMD. The natural history of patients with DMD in Taiwan has not been reported thus far.

Methods: Medical records of 39 patients who received a diagnosis of DMD between 1999 and 2016 at Kaohsiung Medical University Hospital were reviewed. The diagnosis of DMD was confirmed through muscle biopsy or *DMD* genetic analysis.

Results: The mean onset age and mean follow-up period were 2.75 years and 6.76 years, respectively. Seventeen patients (43.5%) had a family history of DMD. The mean full intelligence quotient of the patients was 71.08, and the mean age of walking ability loss was 9.7 years (25 patients). The mean onset age of respiratory insufficiency was 10.64 years with a decline rate of 5.18% per year (25 patients). The mean onset age of cardiomyopathy was 14.69 years (seven patients). The mean onset age of scoliosis was 13.29 years with a

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progression rate of 11.48° per year (14 patients).

Eleven (28.2%) and eight (20.5%) patients had deletions and duplications of *DMD*, respectively. Fourteen patients (35.9%) had point mutations or small deletions or insertions. Five patients received only multiplex ligation-dependent probe amplification (MLPA) analysis and exhibited neither deletion nor duplication. No mutation was identified in one patient through both MLPA and exon sequencing.

Conclusion: The clinical phenotypes and disease course in our cohort were consistent with that reported in previous studies. However, the proportion of point mutations or small deletions or insertions in our study was considerably higher than that in reports from other populations. Cardiac ejection fraction was found not a reliable biomarker for identifying cardiac problems, discovering a better parameter is necessary.

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1. Introduction

Duchenne muscular dystrophy (DMD) is the most common hereditary muscular dystrophy. The prevalence of DMD is approximately 1 in 3800–6300 live male births.^{1–3} DMD is caused by defects in the dystrophin gene (*DMD*), which is responsible for dystrophin production. Dystrophin is an integral part of the dystrophin–glycoprotein complex, which provides structural stability to the skeletal muscles by connecting the sarcolemma and the basal lamina to the inner myofibrillar network by associating with dystroglycan and cytoskeletal proteins. The absence of dystrophin destabilizes muscle membranes and makes them vulnerable to damage during muscle contraction. Different isoforms of dystrophin are derived from different promoters found in the brain, retina, and Purkinje cells. Mutations in these specific isoforms most likely cause extramuscular manifestations such as cognition, behavior, and learning problems.^{4–6}

Typically, patients with DMD become symptomatic between 2 and 5 years of age, and their motor functions deteriorate significantly after the age of 5–7 years through progressive proximal muscle weakness. Patients with DMD usually lose the ability to walk by the age of 12 years. Marked progression of cardiac and respiratory dysfunction is observed during the teenage years. Orthopedic complications and gastrointestinal disturbances are also observed in these patients. In recent years, because of the advancements in cardiac and respiratory interventions, the patients' survival ages have extended to their thirties and forties.^{7,8}

In the past decades, corticosteroid therapy has been reported to improve strength, pulmonary function, and timed motor function as well as reduce the need for scoliosis surgery and delay the onset and progression of cardiomyopathy in patients with DMD.^{9–11} Additionally, comprehensive clinical care guidelines for DMD were proposed by an international, multidisciplinary group of experts and published in 2010, followed by several updated versions.^{12,13} However, no cure for DMD is thus far available although many clinical trials of new therapeutic agents for DMD are underway with some promising results.^{14,15}

This retrospective study analyzed the natural history of patients with DMD and evaluated the effect of steroid therapy, which has never been documented before in Taiwan.

2. Methods

2.1. Patients

We reviewed the medical records of patients who had received a diagnosis of DMD between 1999 and 2016 at Kaohsiung Medical University Hospital. The diagnosis of DMD was confirmed by either muscle biopsy or mutation analysis of *DMD* by using multiplex ligation-dependent probe amplification (MLPA) with or without direct sequencing.

2.2. Study design

Information on the patients' clinical manifestations, serial results of lung function tests (forced vital capacity, FVC), echocardiograms, X-rays of the spine, full intelligence quotient (FIQ) scores, family histories, and genotypes were collected and analyzed. This study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital.

The patients with DMD were divided into two groups, corticosteroid users and nonusers for further analyses. The corticosteroid regimen used in our cohort was prednisolone 0.75 mg/kg on alternate days. Corticosteroid users were defined as patients who had received prednisolone continuously for more than 1 year. Nonusers were defined as patients who had never received corticosteroid treatment or had received the treatment for less than 1 year. The duration of being ambulant, decline in the rate of FVC and left ventricle ejection fraction (LVEF), and rate of scoliosis progress were compared between the two groups. The percentage of predicted FVC calculated from healthy individuals was used for analysis. A patient was considered to exhibit respiratory insufficiency if the FVC was lower than 80% of the predicted value. Data obtained from patients younger than 6 years were excluded because of poor

cooperation. LVEF was measured using the M-mode left ventricle dimensional method on transthoracic echocardiograms. A patient was considered to exhibit cardiomyopathy if the LVEF was less than 50%. The progress of scoliosis was indicated by the change in Cobb's angle in anteroposterior radiographs of the entire spine. For both LVEF and Cobb's angle, a series of data were available after the patients were 8 years old.

2.3. Statistical analysis

Student's *t* test was used for comparing the user and non-user groups. Two-tailed values of $p < 0.05$ were considered statistically significant. The correlation of each parameter with age was analyzed using a linear regression model.

3. Results

3.1. Clinical phenotype

Thirty-nine patients were enrolled in our study. All the patients were Taiwanese, and no significant differences were observed in race or geographic distribution among the patients. The mean onset age and follow-up period were 2.75 years (standard deviation, SD: 2.12) and 6.76 years (SD: 3.59), respectively. The most common initial presentations were falling down easily (43.5%, $n = 17$) and difficulty in climbing stairs or standing up from squatting (28.2%, $n = 11$). Seventeen patients (43.5%) had family histories of DMD. In our study, patients were considered to have a positive family history if they met one of the following criteria: (1) the mother is a DMD carrier; (2) the maternal uncle or maternal male cousin has DMD; or (3) siblings have DMD. The mean age of loss of walking ability was 9.7 years ($n = 25$). The mean age at which respiratory insufficiency was observed was 10.64 years (SD: 3.18), and the decline rate was 5.18% per year ($n = 25$). Because we started prescribing prednisolone regularly to patients with DMD from the age of approximately 6 years, most corticosteroid users did not exhibit cardiomyopathy in our cohort (until 2016). Cardiomyopathy developed in only seven patients, and the mean onset age was 14.79 years. The decline rate was 4.08% per year. The mean onset age of notable scoliosis (Cobb's angle $> 20^\circ$) was 13.29 years with the progression rate of 11.48° per year ($n = 14$). The mean FIQ score of 24 patients who received the cognitive test was 71.08 (SD: 18.2). Among these 24 patients, 10 had FIQ scores < 70 , defined as significant retardation.

3.2. Genotype (Fig. 1)

All 39 patients were subjected to MLPA analysis for mutation identification. Eleven patients (28.2%) had deletions and eight (20.5%) had duplications of *DMD*. Four patients had deletions in the region between exons 45 and 55. The most common deletion occurred between exons 45 and 50 of *DMD* ($n = 4$). Among the remaining 20 patients without deletion or duplication, 15 were subjected to additional direct sequencing analysis, and point mutations or small deletions or insertions were identified in 14 patients (14 in

39, 35.9%). No mutation was observed in one patient through both MLPA and exon sequencing analyses, although the muscle pathology revealed no staining of dystrophin.

3.3. Genotype–phenotype correlation

We further analyzed the patients' data for specific genotype–phenotype correlations. The genotype groups did not differ significantly in ambulatory period and onset age of respiratory or cardiac problems. In our cohort, the mean FIQ score was 64.14 in the point mutation or small deletion or insertion ($n = 7$) group, 78 in the deletion group ($n = 8$), and 67.75 in the duplication group ($n = 4$). Although patients in the deletion group appeared to have higher FIQ scores, no statistically significant difference was observed among the groups.

3.4. Effect of steroid use (Table 1)

In our cohort, 22 patients did not receive corticosteroid treatment or had received the treatment for less than 1 year (six patients were excluded after half of the study period because of poor drug compliance), and 17 patients received prednisolone for more than 1 year. The average duration of corticosteroid use was 4.29 years. The mean ages of loss of walking ability were 9.9 years and 9.51 years in the corticosteroid user ($n = 14$) and nonuser ($n = 11$) groups, respectively. The mean rates of FVC decline were 6.89% per year in the nonuser ($n = 13$) and 0.27% per year in the user ($n = 14$) groups (Fig. 2A and B). Only the data from ages 6 to 15 years were obtained for both groups. The mean rates of LVEF decrease were 0.47% per year in the nonuser ($n = 10$) and 0.06% per year in the user ($n = 13$) groups (Fig. 3A and B). The Cobb's angle in the nonuser ($n = 6$) and user ($n = 3$) groups increased by 13.75° and 4.93° per year, respectively (Fig. 4A and B). For the LVEF and Cobb's angle, only the data from ages 8 to 15 were included for analysis. We compared the data from specific age ranges to reduce the bias of age because, on average, the nonusers were older than the users. However, no statistically significant difference was observed between the two groups in the rates of FVC, LVEF, and Cobb's angle deterioration (Table 1) most likely because of the small number of patients in both groups and the variation among patients resulting from different patterns of home care.

4. Discussion

In this study, the clinical manifestations of our patients, such as initial symptoms and the age of loss of walking ability were similar to those in previous reports from other countries and on other populations.¹⁶ Thus, no significant racial difference was observed in the disease course in the patients with DMD. A genotypic analysis of our cohort revealed that point mutations or small deletions or insertions were the most common mutation patterns, followed by deletion and duplication. The proportion of point mutations or small deletions or insertions (35.9%) was considerably higher than those reported in studies from Oceania (26%), Europe (22%), America (19%), Asia (18%), and Africa (7%).¹⁷ The number might be higher because five

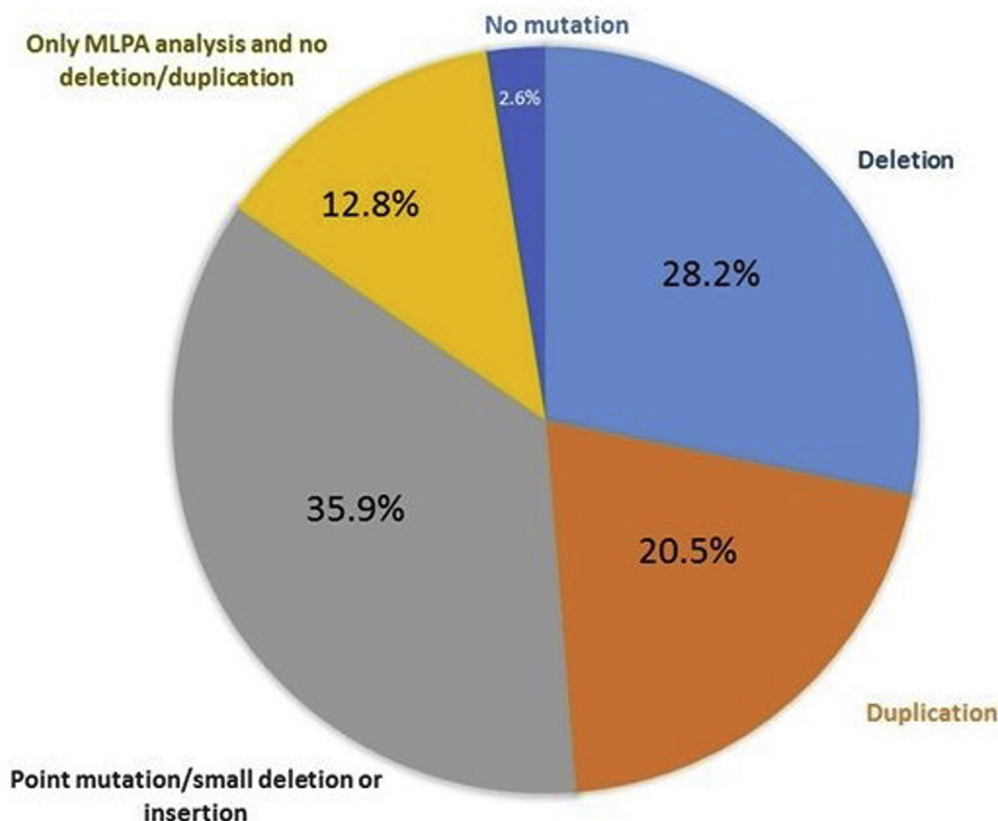


Figure 1 Genotypes of the patients with Duchenne muscular dystrophy.

Table 1 Comparison between corticosteroid users and non-users group.

	Corticosteroid users	Non-users	p-Value
Non-ambulant age (years)	9.51 (n = 14)	9.9 (n = 11)	0.36
Rate of change for predicted FVC (%/year)	-6.89 (n = 13)	-0.27 (n = 14)	0.04
Rate of change for ejection fraction (%/year)	-0.47 (n = 10)	-0.06 (n = 13)	0.85
Rate of change for Cobb's angle (degree/year)	13.75 (n = 6)	4.93 (n = 3)	0.05

patients in our cohort underwent MLPA analysis but not exon sequencing. However, a previous study in Taiwan showed that small mutations accounted for 24.5% (26 in 106) in 92 patients with DMD or Becker muscular dystrophy (BMD) and 14 carriers.¹⁸ Another study on genotypes revealed that MLPA was informative in 60.7% (54 in 89) of the patients with DMD or BMD.¹⁹ The results of these two studies appear to be compatible with data from other populations. The discrepancy between the proportion of small mutations reported in our study and those reported in other Taiwanese studies might have resulted from the small number of patients or other factors such as differences in residential regions and subethnic groups. Additional large-scale studies are necessary to determine the causes of discrepancies.

In our study, no specific genotype–phenotype correlation was observed for FIQ scores. Although the small sample size may have affected the statistical results, overall, the results are consistent with those of other studies. A definite

correlation between the genotype and phenotype of patients with DMD with respect to both the size and pattern of deletion has never been reported.²⁰ A large cohort study identified a tendency toward a lower IQ and earlier wheelchair dependence in patients with distal exon deletions, but the difference was not statistically significant.²¹ Recently, an increasing number of studies have focused on identifying modifier genes toward the natural course of DMD. A study reported that the LTBP4 haplotype affects the age of loss of ambulation in patients with DMD.²² Putative protective effects of a dominant G allele at SPP1 rs28357094 and recessive T allele at LTBP4 rs10880 on the development of cardiac complications in DMD were noted in other studies.²³ The identification of a modifier gene may provide alternative therapeutic strategies in the future.

Of the patients in our cohort, 43% received steroid therapy continuously for more than 1 year. Although a trend showing the benefits of corticosteroid use in delaying the

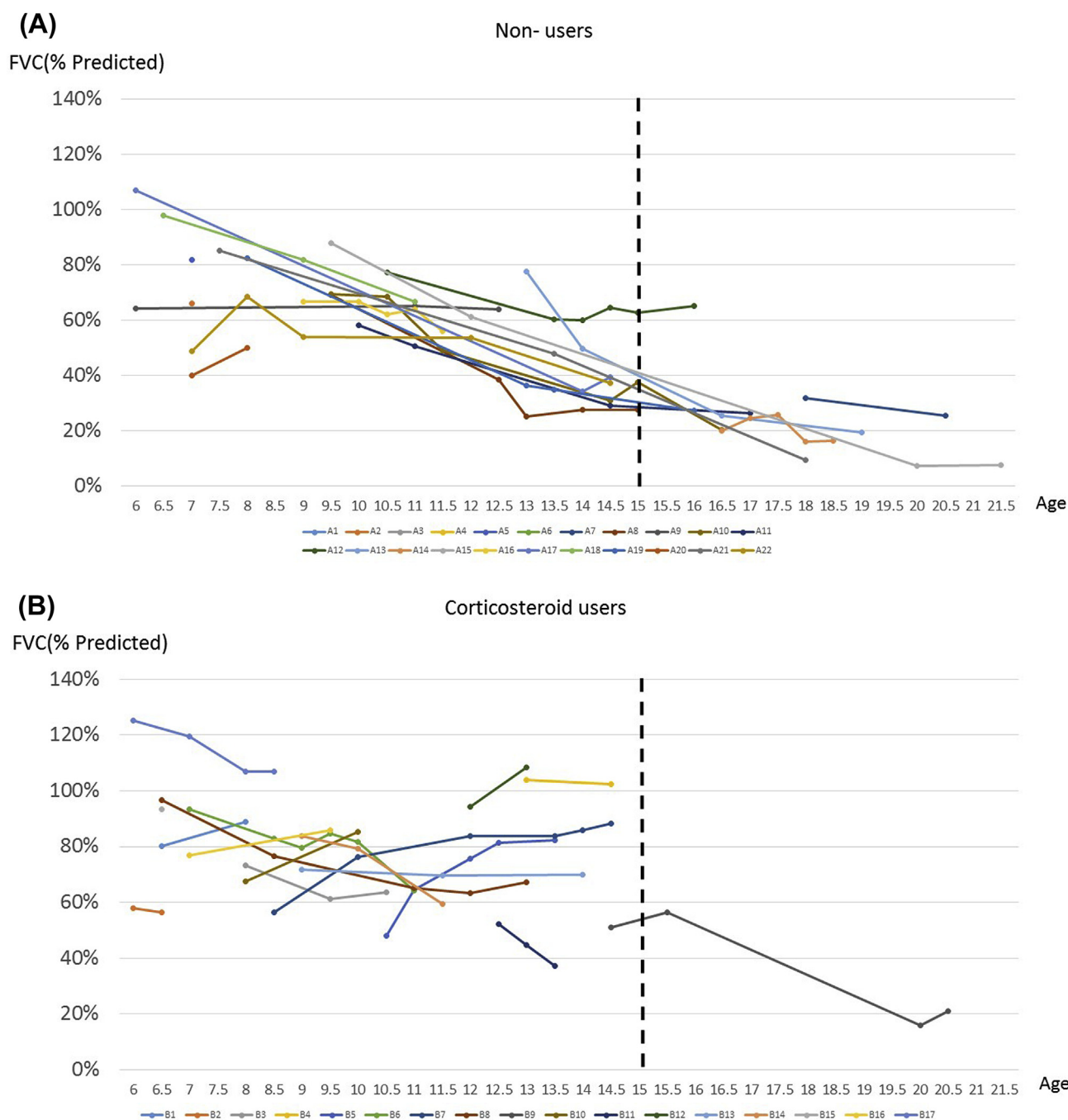


Figure 2 Changes in forced vital capacity in the corticosteroid nonuser (A) and user (B) groups.

deterioration of lung function, cardiac function, and scoliosis was observed, the difference between the times of onset of deterioration between the corticosteroid users and nonusers was not statistically significant. Moreover, no difference was observed in the age of loss of walking ability between the two groups. This result is not consistent with previous reports.²⁴ The most likely cause is that the dosage of steroid used in our cohort (prednisolone 0.75 mg/kg on alternate days) was different from the regimen used in most studies^{11,12,25} (prednisolone 0.75 mg/kg/day).

Moreover, the drug compliance of each patient could not be ensured. Nevertheless, a large-scale trial to determine the optimal therapeutic regimen of corticosteroids for Taiwanese patients is necessary.

In addition to corticosteroid use, multidisciplinary care has become the standard method of management of patients with DMD. It emphasizes the value of multidisciplinary participation and intervention for early detection and prevention of the complications and deterioration of different systems in patients with DMD.¹³ In the past

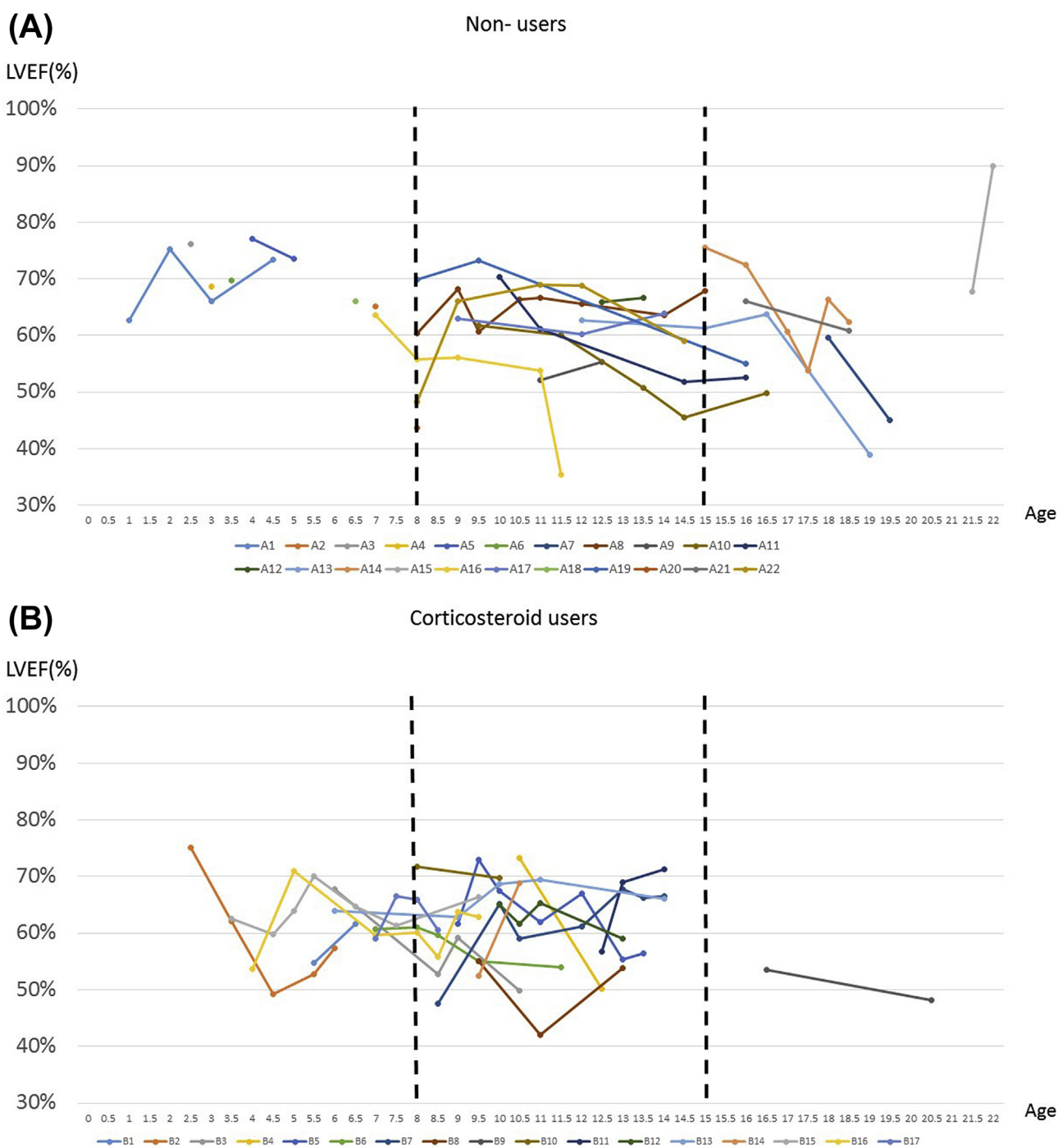


Figure 3 Changes in left ventricle ejection fraction in the corticosteroid nonuser (A) and user (B) groups.

decade, multidisciplinary care has significantly reduced the frequency of hospitalization for commonly observed complications of DMD such as pneumonia, acute respiratory failure, and spine surgery. Most importantly, multidisciplinary care has prolonged the life spans of patients with DMD.²⁶ Because of the small sample size and inadequate tracking time, the effects of multidisciplinary care on the natural history of DMD were not clear in this study. A larger cohort will enable the identification of this effect.

Although no curative therapy for DMD is currently available, many innovative therapeutic approaches are under development.^{27,28} Many countries have prepared to promote clinical research. Establishing a patient database for Taiwan could facilitate international cooperation in a new clinical trial. This study provides the natural history of patients with DMD followed in our hospital, an integrated care center for neuromuscular diseases. We expect that this report will promote related studies at other medical

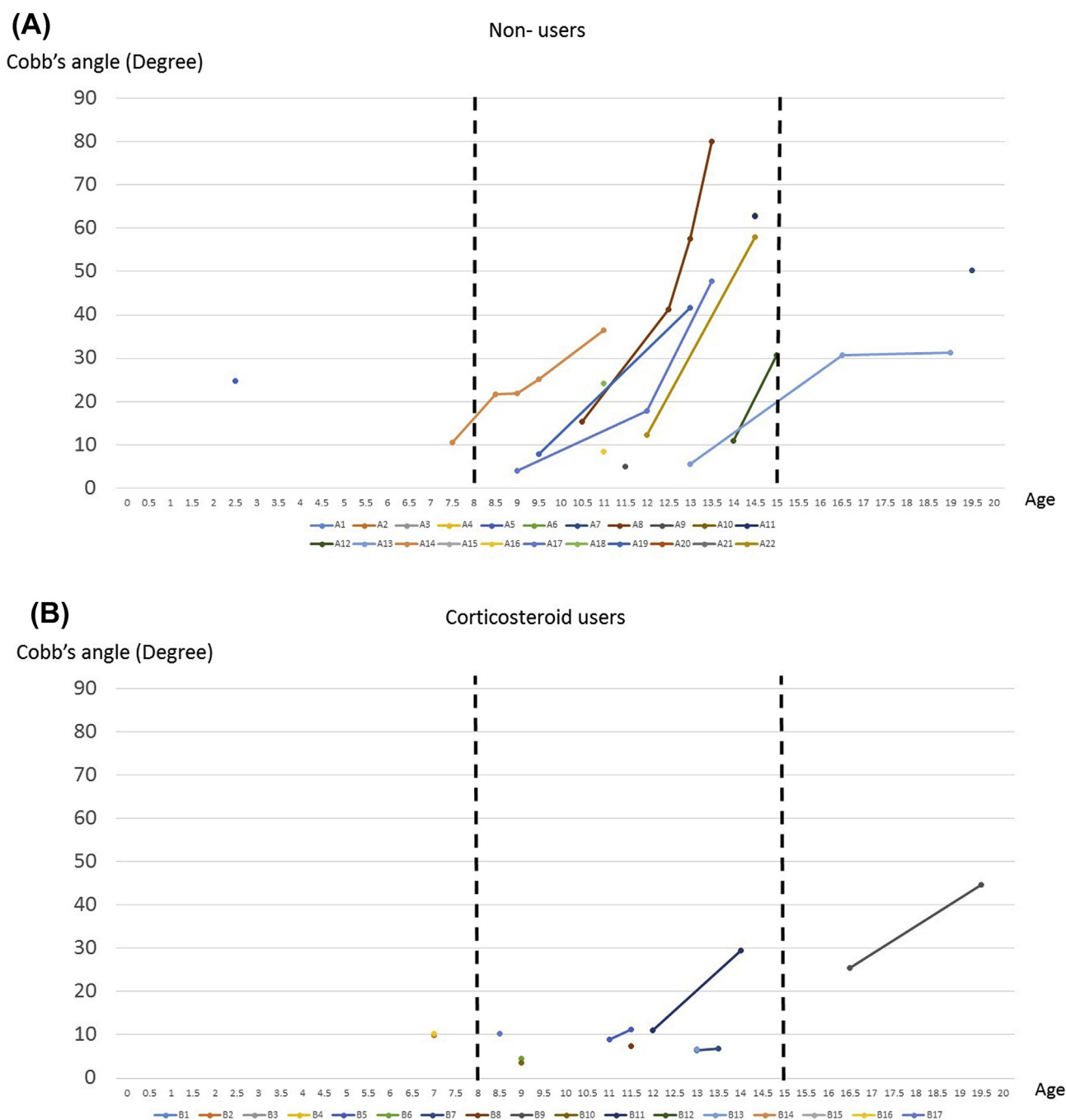


Figure 4 Changes in Cobb's angle in the corticosteroid nonuser (A) and user (B) groups.

institutions, the establishment of a Taiwanese database for DMD, and ultimately, improved care for patients with DMD in Taiwan in the future.

Our study had some limitations. First, we used FVC and LVEF to evaluate lung function and cardiac function. A single parameter may not be sufficient for identifying a functional change. However, verifying new parameters for functional assessment in patients with DMD requires well-designed clinical trials, and a global consensus must be made before applying parameters in patient evaluation. At present, the aforementioned parameters are most commonly used. Second, a limited number of patients and short follow-up period might have affected the results of our study. The functional

deterioration rate might have been underestimated because of the short follow-up period.

Conflicts of interest

No contributing authors have any conflicts of interest to declare.

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