我が国における小児心臓移植の現況

法改正後、18歳未満の方からの臓器提供が2013年12月31日までに6件あり、6名の児童が心臓移植を受けることができた。1名は6歳未満の拡張型心筋症の女児に心臓移植が行われた。児童から心臓移植を受けた患者の心臓移植までの待機期間は成人に比べて短かかった（成人2-3年に対して、小児は1年以内）。しかし、5例目は800日以上の待機後の心臓移植だった。

ドナーが10歳未満で、レシピエントも10歳未満は、2012年6月15日に、6歳未満小児の心臓移植が行われた１例のみ。

国内での心臓移植が非常に困難な10 歳未満の小児を含め、156名が1984 年から2013年末までに海外で心臓移植を受けている。法制定後2011 年12 月末までに海外渡航心臓移植を希望した小児患者（渡航時18 歳未満）は118 人に上り、74 人が心臓移植を受けた（うち8 人は移植後死亡）が、26 人は渡航前に、12 人は渡航後待機中に死亡している。

国内では、2013 年末現在、登録時点で 18 歳未満だった 16 名の児童に対して、心臓移植（成人ドナー10 例）が行われた。拡張型心筋症 14 例、拘束型心筋症 1 例、拡張相肥大型心筋症 1 例で、男児 11 例だった。13 例で移植前にニプロ型補助人工心臓が装着され、2 例がカテコラミン投与、1 例が医学的緊急度 2（入院待機）だった。16 例の待機期間は 182–2026 日(平均 747 日)、補助人工心臓装着期間は 237-1165 日（平均 740 日）だった。1 例が移植後 11 年目に腎不全で死亡したが、他の 15 例は生存中。

児童からの臓器提供

	2011.4.13	2011.9.4	2012.6.15	2013.5.16	2013.8.10	2013.12.7
ドナー年齢	10-15歳	15-18歳	6歳未満	15-18歳	10-15歳	10-15歳
心臓	10代男児（237日）	10代男児（341日）	<10歳女児（267日）	10代女児（264日）	10代男児（865日）	10代女児（871日）
両肺	50代女性	40代女性			30代女性	
肝臓	20代男性	<10歳女児 10代 女児	<10歳女児	60代男性	30代女性	40代男性
膵腎同時	30代女性	30代女性		30代女性 膵単独	40代女性	40代男性
腎臓	60代男性	60代女性	60代女性（2腎）	40代女性	50代男性	40代男性
小腸		30代女性				

（ ）: 待機期間

肺（10施設）※心肺同時移植可能施設

施設番号	施設名	住所	電話番号
R-01	岡山大学病院	〒700-8558 岡山市北区鹿田町2-5-1	086-223-7151
R-02	京都大学医学部附属病院	〒606-8507 京都市左京区聖護院川原町54	075-751-3111
R-03※	大阪大学医学部附属病院	〒565-0871 吹田市山田丘2-15	06-6879-5016
R-04※	東北大学病院	〒980-8574 仙台市青葉区星陵町1-1	022-717-7702
R-05※	独立行政法人国立循環器病研究センター （心肺同時移植のみ）	〒565-8565 吹田市藤白台5-7-1	06-6833-5012
R-06	獨協医科大学病院	〒321-0293 下都賀郡壬生町大字北小林880	0282-86-1111
R-08	福岡大学病院	〒814-0180 福岡市城南区七隈7-45-1	092-801-1011
R-09	長崎大学病院	〒852-8501 長崎市坂本1-7-1	095-819-7200
R-10	千葉大学医学部附属病院	〒260-8677 千葉市中央区亥鼻1-8-1	043-222-7171
R-11	東京大学医学部附属病院	〒113-8655 文京区本郷7－3－1	03-3815-5411

心臓（9施設）※心肺同時移植可能施設

施設番号	施設名	住所	電話番号
S-01※	独立行政法人国立循環器病研究センター（11歳未満移植可能施設）	〒565-8565 吹田市藤白台5-7-1	06-6833-5012
S-02※	大阪大学医学部附属病院（11歳未満移植可能施設）	〒565-0871 吹田市山田丘2-15	06-6879-5016
S-03	東京女子医科大学病院（11歳未満移植可能施設）	〒162-8666 新宿区河田町8-1	03-3353-8111
S-05	東京大学医学部附属病院（11歳未満移植可能施設）	〒113-8655 文京区本郷7－3－1	03-3815-5411
S-06※	東北大学病院	〒980-8574 仙台市青葉区星陵町1-1	022-717-7702
S-07	九州大学病院	〒812-8582 福岡市東区馬出3-1-1	092-641-1151
S-08	北海道大学病院	〒060-8648 札幌市北区北14条西5丁目	011-716-1161
S-09	埼玉医科大学国際医療センター	〒350-1298 日高市山根1397-1	042-984-4111
S-10	岡山大学病院	〒700-8558 岡山市北区鹿田町2-5-1	086-223-7151

Sarcomeric gene mutations and prognosis in pediatric idiopathic cardiomyopathy

Short title: Mutation and treatment outcome in pediatric idiopathic cardiomyopathy

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Abstract

Background: Several genetic abnormalities associated with idiopathic cardiomyopathy have been identified in recent years. However, genotype-phenotype correlation in idiopathic cardiomyopathy remains unclear, especially in childhood. We attempted to assess patients with different types of gene mutations causing pediatric idiopathic cardiomyopathy and investigate the prognosis.

Methods and results: A total of 77 idiopathic cardiomyopathy patients, diagnosed at 16 y of age or when younger, underwent a screening test for myofilament gene mutations by direct sequencing of eight sarcomeric genes, including β-myosin heavy chain (*MYH7*), cardiac myosin-binding protein C (*MyBPC3*), cardiac troponin T (*TNNT2*), cardiac troponin I (*TNNI3*), α-tropomyosin (*TPM1*), regulatory and essential light chains (*MYL2, MYL3*), and cardiac α-actin (*ACTC*). In addition, the clinical presentation and outcomes of the condition in all the patients were evaluated retrospectively. Among the 77 patients screened, patients diagnosed with hypertrophic cardiomyopathy (n = 53), dilated cardiomyopathy (n = 14), restrictive cardiomyopathy (n = 5), and left ventricular noncompaction (n = 5) were included in this study. Patients with a single mutation in *MYH7* (n = 16), *MyBPC3* (n = 6), *TNNT2* (n = 3), *TNNI3* (n = 1), *TPM1* (n = 1), *MYL2*

(n = 1), and patients with mutations in multiple genes (n = 4) were identified. The absence of sarcomeric gene mutations and young age at the time of diagnosis were related to poor prognosis in patients with pediatric idiopathic cardiomyopathy.

Conclusion: Sarcomeric gene mutations and age of the patient at diagnosis are useful predictors of outcome in pediatric idiopathic cardiomyopathy patients.

Key word: idiopathic cardiomyopathy; gene mutation; prognosis

Cardiomyopathies are contractile heart diseases caused by functional abnormalities in cardiomyocytes[1]. In some cases, the patients are susceptible to risk of arrhythmias and sudden cardiac death[2]. Cardiomyopathies are mainly classified as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and unclassified cardiomyopathies including left ventricular noncompaction (LVNC)[3]. In these cardiomyopathies, more than 50 genes have been identified as disease-causing genes within the last two decades[4]. Mutations in genes that encode sarcomeric proteins are as follows: β-myosin heavy chain (*MYH7*, OMIM *160760), cardiac myosin binding protein (*MyBPC3*, OMIM *600958), cardiac troponin T (*TNNT2*, OMIM*191045), cardiac troponin I (*TNNI3*, OMIM*191044), α-tropomyosin (*TPM1*, OMIM*191010), regulatory and essential light chains (*MYL2*, OMIM*160781 and *MYL3*, OMIM*160790), and actin (*ACTC1*, OMIM*102540) are recognized as the primary causatives of HCM.[5,6] Some of these genes are also known to be associated with DCM and RCM pathogenesis[4,7]. Therefore, there probably exits a common genetic basis for these cardiomyopathies.

Some previously published reports have focused on the epidemiology of

pediatric cardiomyopathy.[8,9] However, the clinical outcomes associated with each of the gene mutations identified in pediatric idiopathic cardiomyopathies remains unclear. Although, several studies suggest a genetic correlation in idiopathic cardiomyopathies[2,6,10-16], further investigation is required to make a firm and convincing conclusion.

In this study, we attempted to screen for disease-causing sarcomeric genes and conducted a follow-up survey to clarify the clinical characteristics and interrelationship between the gene mutation and prognosis in patients with pediatric cardiomyopathy.

Methods

Study Population

Idiopathic cardiomyopathy patients (n = 77), diagnosed at ≦15 y of age were selected from 45 hospitals in Japan. Care was taken to avoid duplication of patients during the selection process. Cardiomyopathy was diagnosed through clinical evaluation, chest radiography, electrocardiography, echocardiography, and cardiac catheterization based on current international consensus criteria. Patients with any other complications, cardiac or systemic disease attributable to cardiomyopathy were excluded from this study by trained cardiologists.

Some of these patients have been described in previous reports[16]. Written informed consent was obtained from guardians of all the patients in accordance with the Declaration of Helsinki. We retrospectively assessed each patient by clinical history, physical examination, current therapy, and a review of their medical records until March, 2013.

Genetic Study

The coding regions and exon-intron boundaries of eight sarcomeric genes, *MYH7*, *MyBPC3*, *TNNT2*, *TNNI3*, *TPM1*, *MYL2*, *MYL3*, and *ACTC* were amplified from the genomic DNA of individual patients included in the study. Amplified products were purified using the QIAquick polymerase chain reaction (PCR) purification method (QIAGEN, Hilden, Germany) and screened using bi-directional direct sequencing with an ABI 3130xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). When a novel mutation was detected, we confirmed its absence in more than 100 healthy controls by direct sequencing.

Statistical Analysis

Clinical features were expressed as mean ± standard deviation (SD), median (interquartile range) or number of patients (percentage), as appropriate. Univariate comparisons were performed using the Student's *t*-test for continuous measures and the chi-square test for categorical measures.

A Kaplan-Meier overall survival curve was constructed to demonstrate the overall survival difference between mutation groups and compared using the log-rank test. Cox proportional hazards regression model was used to examine the relationship between mutations and all-cause death. Similar survival analyses were performed for genders, family history of sudden death, family history of cardiomyopathy, or arrhythmia.

A p value of <0.05 was considered significant. Statistical analyses were performed using JMP for Windows (version 10; SAS Institute, North Carolina, USA).

Results

Seventy-seven patients with pediatric idiopathic cardiomyopathy, corresponding to 36 men and 41 women, were included in this study (Table 1). The average age at the

time of diagnosis was 8.8 y (quartile range: 1.2–12.6). Fifty-three (68.8 %) were HCM, 14 (18.2 %) were DCM, 5 (6.5 %) were RCM, and 5 (6.5 %) were LVNC. There were no patients with ARVC and unclassified cardiomyopathy, excluding LVNC.

Among those, 32 (41.6%) patients carried a mutation in the disease-causing sarcomeric gene. Mutations in *MYH7, MyBPC3,* and *TNNT2* genes were identified in 16 (20.8%), 6 (7.8%), 3 (3.9%) patients, respectively. Mutations in *TNNI3, TPM1*, and *MYL2* genes were identified in 1 (1.3%) patient each (Table 1). In addition, 4 HCM patients (5.2%) possessed multiple mutations, with three of them harboring double mutations in *MyBPC3* and the other patient with mutations in both *MYH7* and *MyBPC3* genes. The rest of the patients did not have mutations in any of the genes tested (n = 45), and *ACTC* mutation was not detected. The relationship between gene mutation and baseline characteristics are outlined in Table 2.

Sarcomeric Gene Mutation Noncarrier is a Risk Factor of Poor Treatment Outcome

The median follow-up duration was 6.8 y (quartile range 1.4–15.3). Twelve (15.6 %) patients died, and in all these cases, the idiopathic cardiomyopathy was the primary cause of death (Table 1). We investigated some characteristics of these patients

to find good predictors of treatment outcome in patients with pediatric idiopathic cardiomyopathy. We found no significant differences between outcome and gender, family history of sudden death, family history of idiopathic cardiomyopathy, and arrhythmia (data not shown). We could find, however, that the prognosis of patients carrying mutations in *MYH7, MyBPC3, TNNT2, TNNI3, TPM1*, or *MYL2* was significantly better than that in the patients lacking mutations (Figure 1. Log-rank test $p = 0.034$). A multivariate Cox proportional-hazards model for the time to death also showed a finding similar to that of the Log-rank test (Figure 1. hazard ratio, 4.51; 95% confidence interval, 1.18–29.37; $p = 0.026$).

The Combination of Age at Diagnosis and the Identification of Sarcomeric Gene Mutation/s is a Useful Predictor of Prognosis in Pediatric Idiopathic Cardiomyopathy.

Based on the above result, we considered baseline characteristics in two categories, the mutation-positive group, constituting patients with mutations in any of the five disease causing genes and the mutation-negative group, lacking mutations. As shown Table 3, the age at diagnosis in the mutation-negative group was significantly lower than that in the mutation-positive group ($p = 0.004$).

As indicated by the results in Table 3, we assessed the relationship between age at diagnosis and overall survival. Using the median age at diagnosis (8.8 y), we divided the patients into 2 groups and compared the outcome. A multivariable Cox proportional hazards model for time to death indicated that the group of lower age at diagnosis (<8.8 y) had a significantly worse outcome than the group of higher age at diagnosis (≧8.8 y) (Figure 2. log-rank test $p = 0.015$). Cox proportional hazards model for mortality also indicated that the lower age at diagnosis was strongly associated with poor outcome (hazard ratio, 5.37; 95% confidence interval, 1.41–34.94; $p = 0.011$).

Besides, we investigated whether age at diagnosis would have an influence on the outcome of the patients with disease-causing mutations in 5 genes. First, we stratified all the patients into 4 groups according to the median age at diagnosis and based on the presence or absence of the 5 disease-causing gene mutations. Secondly, we assessed the clinical outcome of each group using the Kaplan-Meier curves. Patients with a lower age at diagnosis and no mutation in any of the 5 disease-causing genes showed significantly worse prognosis among these 4 groups (log-rank test $p = 0.024$; Figure 3).

Patients with Truncating Mutations Have More Severe Disease than Those with Missense Mutations or Small Deletions

Finally, we focused specifically on the clinical outcome associated with the disease-causing mutations in the 5 genes. Table 4 summarizes the details on each gene mutation[17-37]. Some mutations listed in this study has been previously reported by our colleagues[16]. In this study, we identified 4 novel *MYH7* mutations and 3 novel *MyBPC3* mutations. We could not find any differences in the clinical outcome among these 5 gene mutations (data not shown), but it was notable that patients with truncation mutations had poorer prognosis than patients with missense mutation or small deletion (log-rank test $p = 0.005$; Figure 4).

Discussion

To our knowledge, this is the first study attempting to identify predictors of prognosis in pediatric idiopathic cardiomyopathy as a whole. Thus far, there have been only a few reports on the clinical outcome in pediatric idiopathic cardiomyopathy. Recently, Alexander et al investigated the outcome of DCM diagnosed during childhood and revealed that age at diagnosis, familial cardiomyopathy, and severity of left

ventricular dysfunction are risk factors for death or heart transplantation[38]. Our observations are contrary to that report, and here, we have conducted a comprehensive evaluation of prognosis of pediatric idiopathic cardiomyopathy by including genetic mutations associated with multiple cardiomyopathies[4,39]. Genetic analysis is an essential parameter and is justified by the common pathophysiological mechanism observed in HCM and DCM, with some of the HCM cases progressing to DCM.

In this study, we revealed that patients with disease causing mutations in 5 sarcomeric genes had better prognosis than those without mutations. The reason for this result is unclear. There were no significant differences between outcome and family history of sudden death, family history of idiopathic cardiomyopathy, but it is possible that early genetic counseling for patients with idiopathic cardiomyopathy and their relatives might have an effect on the prognosis to some degree. It is also to be considered that idiopathic cardiomyopathy is caused by mutations in more than 50 genes[4], although the scope and the outcomes explained in this study is limited to only 8 sarcomeric genes. We chose these major genes based on their importance and high mutation frequency in idiopathic cardiomyopathy patients. At this point, there are no correlations or noteworthy characteristics associating the disease-causing mutations in any other genes with prognosis. However, the possibility of unknown gene mutations and their effect on

prognosis cannot be ruled out. In addition, among the unknown gene mutations, those in genes unrelated to currently known disease-causing genes might have a high impact on the pathogenesis of idiopathic cardiomyopathy.

Although, there are few papers in which the relationship between sarcomeric gene mutations and prognosis in idiopathic cardiomyopathies as a whole is mentioned, Xu et al reported that patients with a homozygous mutation or two or more heterozygous mutations often show more severe phenotypes[1]. In addition, Walsh et al advocated that changes in amino acid composition have an important role in determining the severity in case of cardiomyopathy patients with *MYH7* mutation[2]. However, we did not find any differences among the outcomes in each gene mutation carriers (data not shown). Besides, the number of mutation changes in amino acid composition did not influence the clinical outcome (data not shown). This result may be due to the small sample size and short follow-up period in this study. A larger patient population needs to be included and continued follow-up needs to be performed to find some predictors associated with the type of gene mutation.

In this study, we also revealed that young age at diagnosis was a risk factor for poor prognosis in patients with pediatric idiopathic cardiomyopathy. Colan reported that patients with idiopathic HCM diagnosed before 1 y of age had more severe

prognosis than those diagnosed after 1 y of age[39]. On the other hand, Alexander et al, assessed risk factors of DCM in patients, whose diagnosis age was between 0 and 10 y, and they observed that age of less than 4 weeks or more than 5 y at time of diagnosis was associated with increased risk of death or transplantation requirement[38]. The relationship between age at diagnosis and clinical outcome of pediatric idiopathic cardiomyopathies as a whole has not been reported, and therefore, it is difficult to assess our current results in comparison with previous reports. Nevertheless, clinical family screening for infants and young children may still be useful. Although Maron et al suggested that periodic clinical screening of family members of HCM patients who are under 12 y is not necessary, unless they lack (i) malignant family history, (ii) any symptoms, (iii) any opportunity as competitive athletes, or (iv) other clinical suspicion of early left ventricular hypertrophy[41], in which case, it might be important to check their state of health regularly, regardless of such conditions.

We also revealed that pediatric idiopathic cardiomyopathy patients with truncating mutations had poorer prognosis than those with missense mutations or small deletions. The reason underlying this novel observation is not clear, but Erdmann et al. reported that truncation mutations of *MyBPC3* seemed to cause a more severe disease phenotype than missense mutations or in-frame deletions of *MyBPC3* in HCM patients[42].

The mechanism of *MyBPC3* mutation may be more common than that of other disease-causing genes. Further studies should be performed to investigate the relation between mutation type and clinical outcomes.

Conclusions

Sarcomeric gene mutation and age at diagnosis are valuable predictors of prognosis in patients with pediatric idiopathic cardiomyopathy. In addition, patients with truncating mutations have poorer prognosis than those with missense mutations or small deletions. These results will provide practical benefit for patients with pediatric idiopathic cardiomyopathy and young family members of idiopathic cardiomyopathy patients.

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Disclosures

None.

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