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Figure Legends

Figure 1. Survival of pediatric idiopathic cardiomyopathy patients with disease-causing sarcomere gene mutations (Mutation positive) and those lacking mutations (Mutation negative) (log-rank test, $p = 0.034$).

Figure 2. Time to death in all patients according to age at the time of diagnosis. Patients were grouped based on the median age at diagnosis (8.8 y; log-rank test, $p = 0.015$).

Figure 3. Overall survival in all the patients according to age at diagnosis and disease-causing sarcomere gene mutation (log-rank test, $p = 0.024$).

Figure 4. Influence of disease-causing sarcomere gene mutation types on prognosis of patients with pediatric idiopathic cardiomyopathy (log-rank test, $p = 0.005$).

Table 1. Baseline charactaristics of 77 childhood cardiomyopathy patients

Variable	All patients (n = 77)
Male / Female	36 / 41
Age at diagnosis (year)	8.8 (quartile range 1.17 - 12.6)
Follow-up duration (year)	6.8 (quartile range 1.35 - 15.3)
Type of IM	
HCM	68.8 % (53 / 77)
DCM	18.2 % (14 / 77)
RCM	6.5 % (5 / 77)
noncompaction	6.5 % (5 / 77)
Family history of IM	54.5 % (42 / 77)
Family history of sudden death	26.0 % (20 / 77)
Brain natriuretic peptide (pg/ml)	330.2 (quartile range 33.8 - 860.6)
Arrhythmia	19.5 % (15 / 77)
Disease causing gene mutation	41.6 % (32 / 77)
MYH7	20.8 % (16 / 77. 14 HCM, 1 DCM, 1 RCM)
MyBPC3	7.8 % (6 / 77. 5 HCM, 1 DCM)
TNNT2	3.9 % (3 / 77. 3 HCM)
TNNI3	1.3 % (1 / 77. 1 RCM)
TPM1	1.3 % (1 / 77. 1 noncompaction)
MYL2	1.3 % (1 / 77. 1 HCM)
MYL3	0%
ACTC	0%
Multiple mutation	5.2 % (4 / 77. All 4 patients are HCM. 3 *MyBPC3* double mutation carriers and 1 *MYH7* and *MyBPC3* mutation carrier)
Prognosis	
NYHA class I	26
NYHA class II	18
NYHA class III	3
NYHA class IV	1
Heart transplantation	3
Death	12
Unknown	14

ACTC, actin; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; IM, idiopathic cardiomyopathy; MyBPC3, cardiac myosin binding protein; MYH7, β-myosin heavy chain; MYL, essential light chains; NYHA, New York Heart Association classification; RCM, restrictive cardiomyopathy; TNNI3, cardiac troponin I; TNNT2, cardiac troponin T; TPM1, α-tropomyosin

Table 2. Baseline characteristics and gene mutations

Genes	Median age at diagnosis (quartile range)	Gender (M / F)	Median duration of follow-up, y (quartile range)
βMHC	12.1 y (11.2 - 13y)	7 / 9	7.25 (1 - 20.4)
MyBPC	4.4 y (0.4 - 10.5y)	2 / 4	14.8 (8.1 - 17.4)
TNNT2	14.9 y (12.6 - 15.6y)	1 / 2	10 (8.3 - 14.7)
TNNI3	6.6 y	1 / 1	3.2
TPM1	8.1 y	0 / 1	0.6
MYL2	11.3	1 / 0	5.9
Multiple mutation	12.6 y (9.6 - 13y)	2 / 2	19.3 (6.8 - 23)
Mutation negative	4.8 y (0.4 - 12.3y)	23 / 22	5.1 (0.5 - 11.9)

MyBPC3, cardiac myosin binding protein; MYH7, β-myosin heavy chain; MYL, essential light chains; TNNI3, cardiac troponin I; TNNT2, cardiac troponin T; TPM1, α-tropomyosin.

Table 3. Comparison of baseline characteristics between mutation negative patients and mutation positive patients

Variable	Mutation negative (n=45)	Mutation positive (n=32)	*p* value
Male / Female	23 / 22	13 / 19	0.363
Age at diagnosis (year)	4.8 (quartile range 0.5-12.3)	12.0 (quartile range 8.2-13.0)	0.004
Follow-up duration (year)	5.1 (quartile range 0.5-11.85)	9.6 (quartile range 3.23-17.4)	0.068
Family history of IM	44.4% (22/45)	68.8% (22/32)	0.052
Family history of sudden death	20.0% (9/45)	34.4% (11/32)	0.089
Arrhythmia	15.6% (7/45)	25.0 (8/32)	0.293
Prognosis			
NYHA I	13	13	0.162
NYHA II	9	9	
NYHA III	2	1	
NYHA IV	0	1	
Heart	3	0	
Death	10	2	
Unknown	8	6	

IM, idiopathic cardiomyopathy; NYHA, New York Heart Association class

Table 4. Details of mutations with childhood idiopathic cardiomyopathy patients

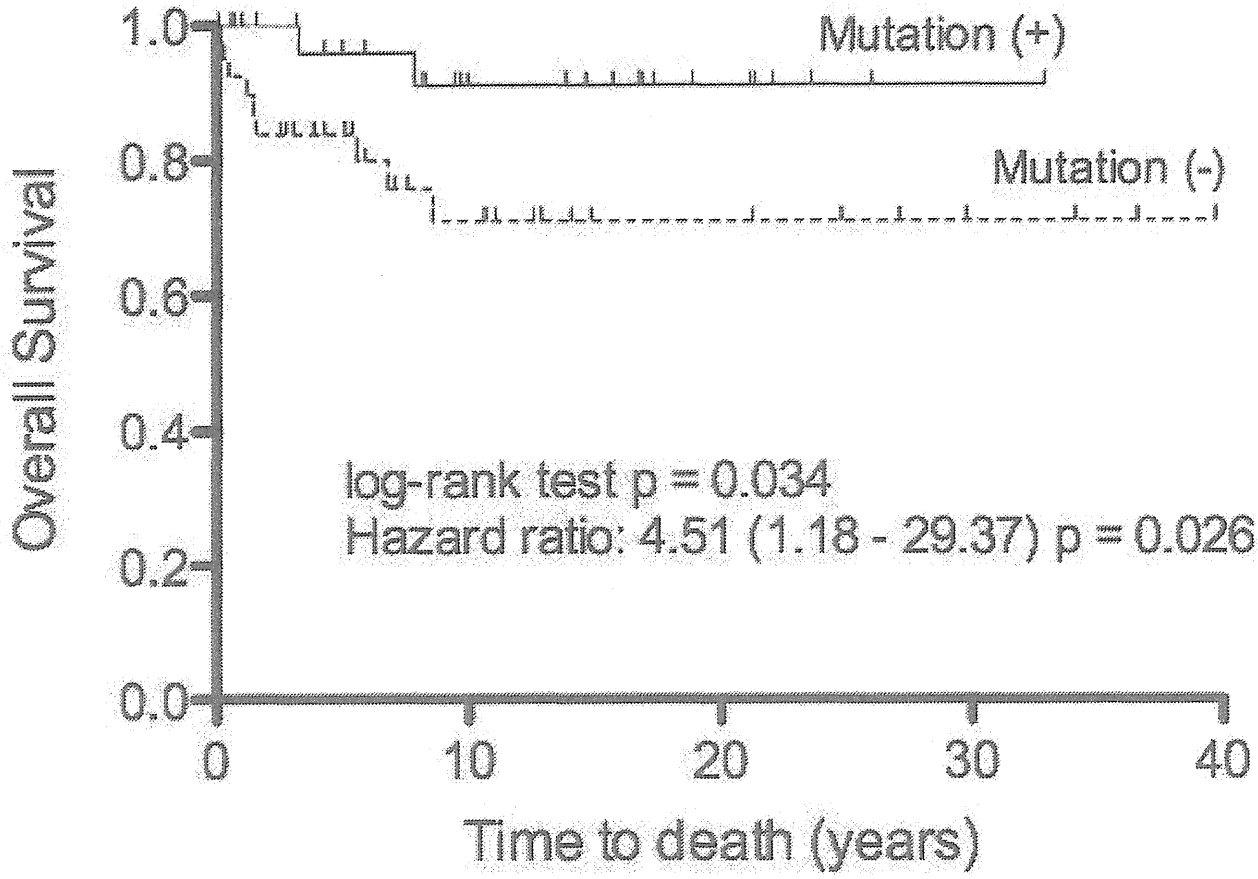
Single mutation

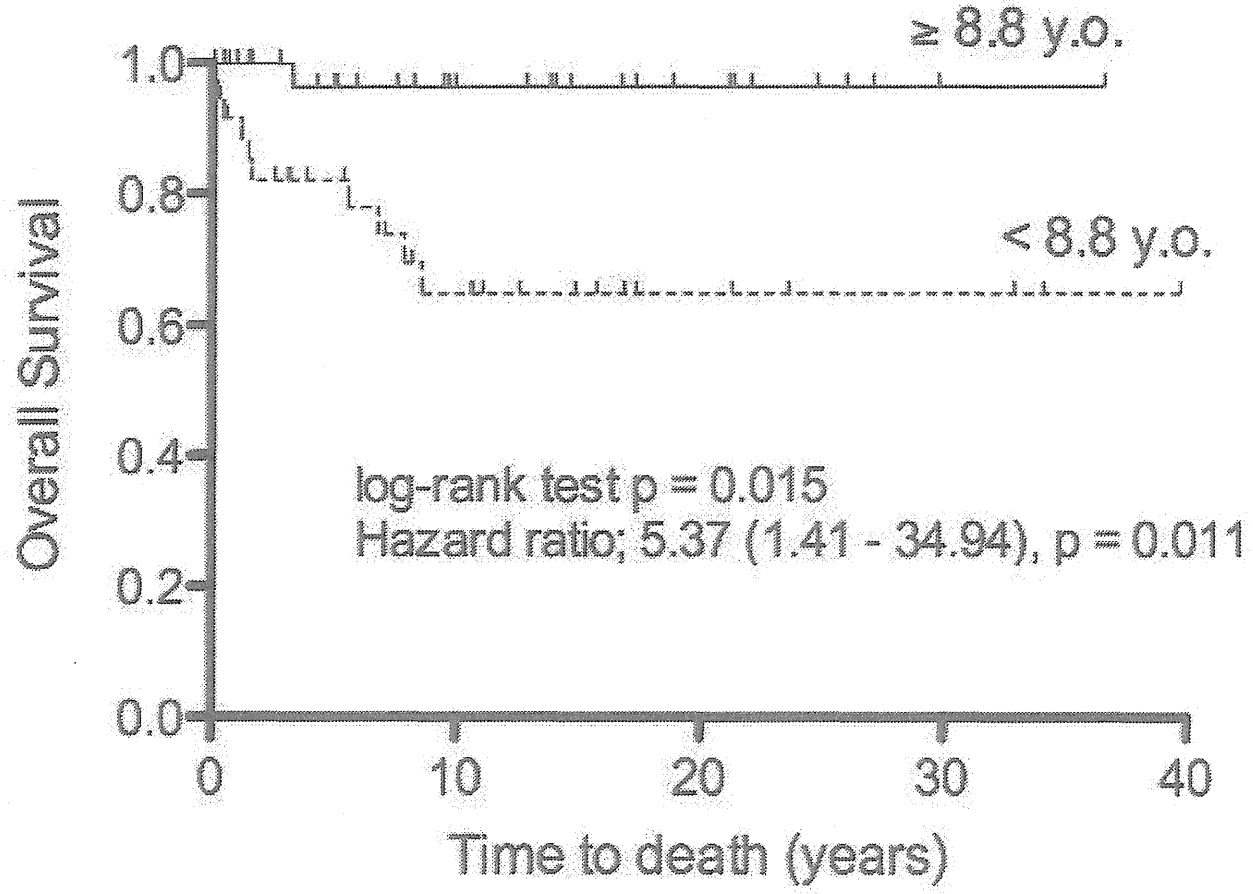
Gene	Proband	Mutation Location	Mutation Category	Domain	Nucleotide Change	Amino Acid Change	Reference
MYH7	1	Exon 12	Missense		c.1012 G>A	p.V338M	This study
	2	Exon 14	Missense		c.1327 A>T	p.I443F	This study
	3	Exon 18	Missense		c.1987 C>T	p.R663C	Driest et al.[17]
	4	Exon 18	Missense		c.2011 C>T	p.R671C	Richard et al.[18]
	5	Exon 19	Missense		c.2155 C>T	p.R719W	Anan et al.[19]
	6	Exon 19	Missense		c.2156 G>A	p.R719Q	Consevage et al.[20]
	7	Exon 20	Missense		c.2221 G>T	p.G741W	Arai et al.[21]
	8	Exon 20	Missense		c.2221 G>T	p.G741W	Arai et al.[21]
	9	Exon 21	Small deletion		c.2302_2310 del GGGCTGCTG	p.768_770 del G,L,L	This study
	10	Exon 21	Small deletion		c.2302_2310 del GGGCTGCTG	p.768_770 del G,L,L	This study
	11	Exon 21	Missense		c.2333 A>G	p.D778G	Harada et al.[22]
	12	Exon 22	Missense		c.2596 T>C	p.S866P	This study
	13	Exon 22	Missense		c.2609 G>A	p.R870H	Watkins et al.[23]
	14	Exon 23	Missense		c.2770 G>A	p.E924K	Watkins et al.[24]
	15	Exon 23	Missense		c.2770 G>A	p.E924K	Watkins et al.[24]
	16	Exon 23	Missense		c.2803 G>A	p.E935K	Nishi et al.[25]
MyBPC3	1	Exon 19	Frameshift		c.1777 del T	p.S593fs	This study
	2	Exon 19	Missense		c.1790 G>A	p.R597Q	Berge et al.[26]
	3	Exon 22	Missense		c.1976 T>C	p.I659T	This study
	4	Exon 28	Nonsense		c.2827 C>T	p.R943X	Alders et al.[27]
	5	Exon 28	Frameshift		c.2833_2834 del CG	p.R945fs	Anan et al.[28]
	6	Exon 29	Missense		c.2992 C>G	p.Q998E	Driest et al.[29]
TNNT2	1	Exon 8	Missense		c.236 T>C	p.I79T	Fujita et al.[16]
	2	Exon 8	Missense		c.274 C>T	p.R92W	Moolman et al.[30]
	3	Exon 10	Missense		c.388 T>C	p.R130C	Song et al.[31]
TNNI3	1	Exon 8	Missense		c.575 G>A	p.R192H	Mogensen et al.[32]
TPM1	1	Exon 1	Missense		c.109 A>G	p.K37E	Chang et al.[33]
MYL2	1	Exon 4	Missense		c.173 G>A	p.R58Q	Flavigny et al.[34]

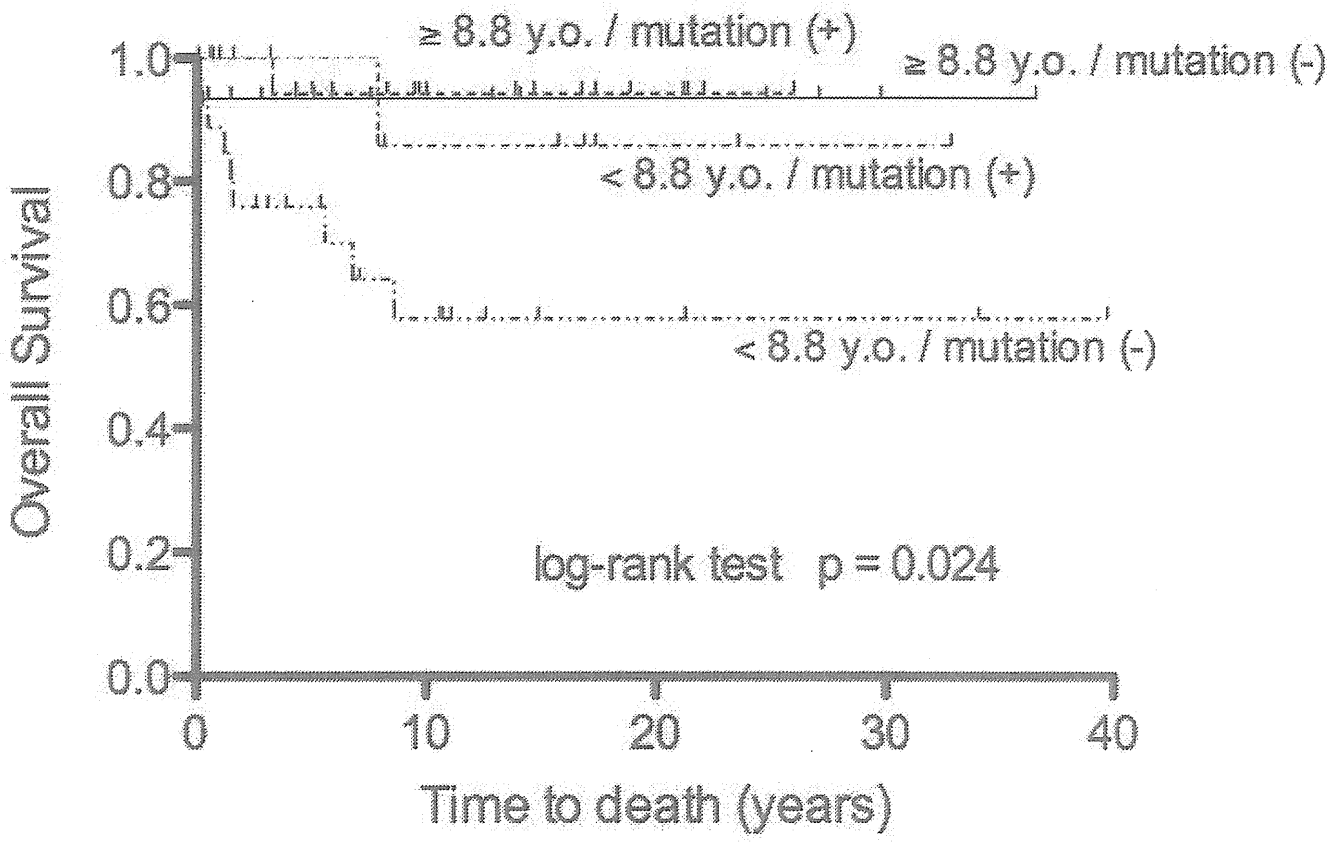
Multiple mutations

Proband	Gene	Mutation Location	Mutation Category	Domain	Nucleotide Change	Amino Acid Change	Reference
1	*MYH7*	Exon 14	Missense		c.1357 C>T	p.R453C	Watkins et al.[24]
	MyBPC3	Exon 29	Missense		c.2992 C>G	p.Q998E	Driest et al.[30]
2	*MyBPC3*	Exon 13	Missense		c.1000 G>A	p.E334K	Anan et al.[35]
	MyBPC3	Exon 18	Missense		c.1594 C>T	p.R502W	Richard et al.[18]
3	*MyBPC3*	Exon 17	Missense		c.1451 T>A	p.V484D	This study
	MyBPC3	Exon 28	Frameshift		c.2833_2834 del CG	p.R945fs	Anan et al.[29]
4	*MyBPC3*	Exon 10	Nonsense		c.890 C>G	p.S297X	Hirota et al.[36]
	MyBPC3	Exon 18	Missense		c.1519 G>A	p.G507R	Erdmann et al.[37]

MyBPC3, cardiac myosin binding protein; MYH7, β-myosin heavy chain; MYL, essential light chains; TNNI3, cardiac troponin I; TNNT2, cardiac troponin T; TPM1, α-tropomyosin.


Mutation (+)
Mutation (-)
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
log-rank test p = 0.034
Hazard ratio: 4.51 (1.18 - 29.37) p = 0.026
0
10
20
30
40
Time to death (years)


≥ 8.8 y.o.
< 8.8 y.o.
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
log-rank test p = 0.015
Hazard ratio; 5.37 (1.41 - 34.94), p = 0.011
0
10
20
30
40
Time to death (years)


≥ 8.8 y.o. / mutation (+)
≥ 8.8 y.o. / mutation (-)
< 8.8 y.o. / mutation (+)
< 8.8 y.o. / mutation (-)
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
log-rank test p = 0.024
0
10
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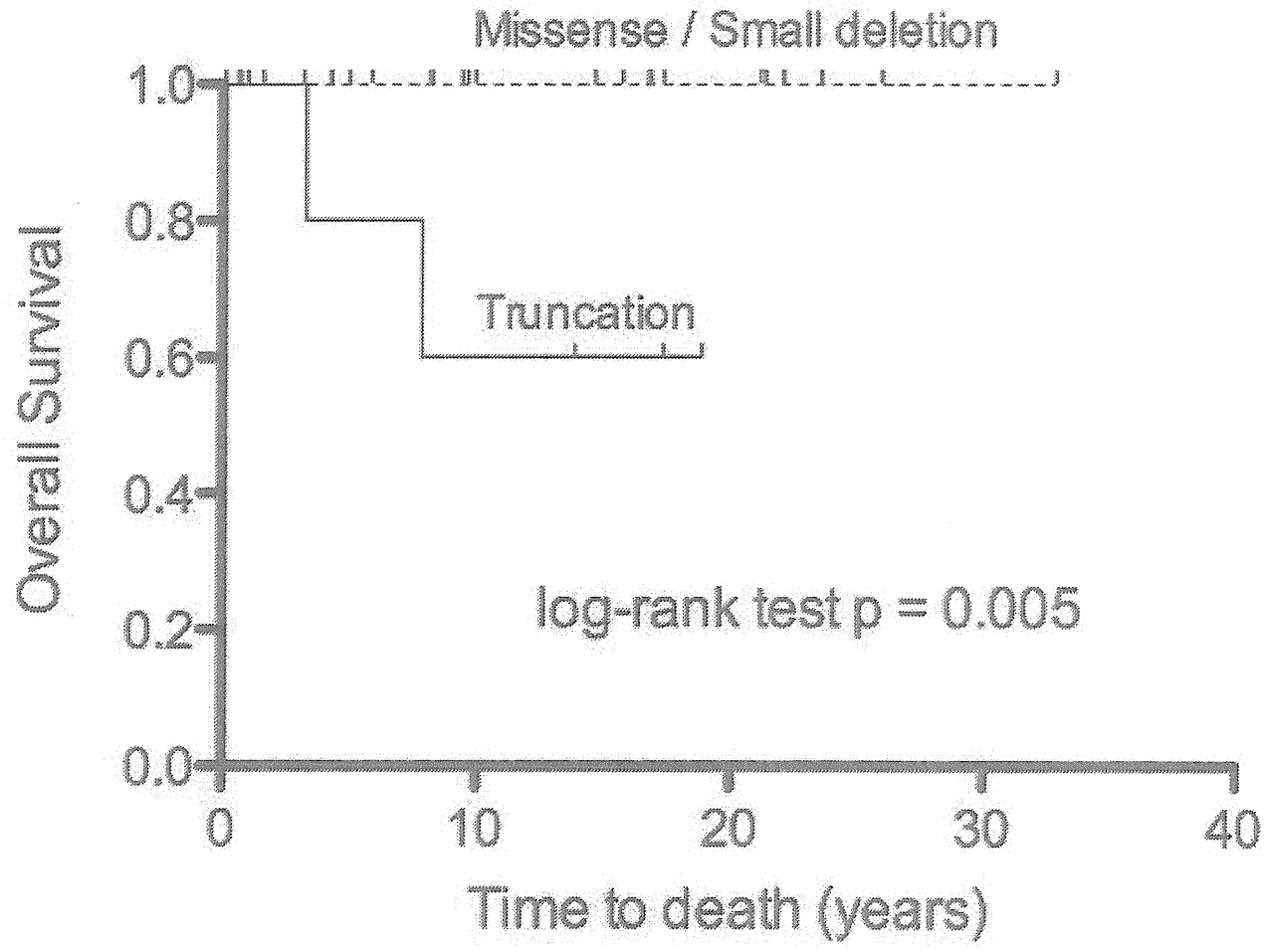
Time to death (years)


Missense / Small deletion
Truncation
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
log-rank test p = 0.005
0
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Time to death (years)

Heart Vessels
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ORIGINAL ARTICLE

Mutations in the cardiac troponin T gene show various prognoses in Japanese patients with hypertrophic cardiomyopathy

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Abstract Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disorder resulting from mutations in genes for at least 15 various sarcomere-related proteins including cardiac β-myosin heavy chain, cardiac myosin-binding protein C, and cardiac troponin T. The troponin T gene (*TNNT2*) mutation has the third incidence of familial HCM, and the genotype–phenotype correlation of this gene still remains insufficient in Japanese familial HCM. Therefore, in the present study, we focused on screening the *TNNT2* mutation in 173 unrelated Japanese patients with familial HCM, and found three reported mutations and a new mutation of *TNNT2* in 11 individuals from four families. In these families, two individuals from one family had double mutations, Arg130Cys and Phe110Ile, six individuals from two other families had an Arg92Trp mutation, and one individual of another family had a new mutation, Ile79Thr, of *TNNT2*. The phenotype of each family was often different from reported cases, even if they had the same genetic mutation. In addition, families with the same genetic mutation showed a similar trend in the phenotype, but it was not exactly the same. However, sudden death in youth was observed in all of these families. Although the type of genetic mutation is not useful for predicting prognosis in HCM, the possibility of sudden cardiac death remains. Therefore, the prognosis of individuals bearing the *TNNT2* mutation with familial HCM should be more carefully observed from birth.

Keywords Familial hypertrophic cardiomyopathy · *TNNT2* gene · Mutation · Phenotype–genotype

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Introduction

Hypertrophic cardiomyopathy (HCM) is characterized by left and/or right ventricular hypertrophy, with predominant involvement of the interventricular septum in the absence of other causes of hypertrophy, such as hypertension, valvular heart disease, or metabolic disease [1–7].

Familial HCM is an autosomal dominant disorder caused by mutations in genes that encode sarcomere proteins. It has been reported that at least 15 genes are implicated in 55–70 % of HCM, and the major genes causing HCM include cardiac β-myosin heavy chain (*MYH7*), α-tropomyosin, cardiac troponin T (*TNNT2*), cardiac myosin-binding protein C (*MYBPC3*), cardiac troponin I (*TNNI3*), cardiac myosin regulatory light chains (*MYL2*), and cardiac myosin essential light chains (*MYL3*) [1, 4, 5, 8–11].

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Cardiac troponin T is a thin-filament protein that takes part in muscle contraction. The troponin complex on the actin filament regulates the force and velocity of muscle contraction. Troponin C functions as a calcium receptor while troponin I inhibits adenosine triphosphatase (ATPase) activity when bound to actin. Troponin T fixes the troponin group to tropomyosin. During relaxation, the troponin group is bound to actin and tropomyosin, blocking the interaction of myosin and actin [12].

TNNT2 was mapped to chromosome 1q32. Mutations of *TNNT2* were thought to account for approximately 15 % of familial HCM, with most missense mutations located in exons 8–16 [3], and were associated with a particularly severe form of the disease characterized by a poor overall prognosis with a high incidence of sudden death despite only mild left ventricular hypertrophy (LVH) [1–4]. Most *TNNT2* mutations of familial HCM alter the contractile properties of cardiac muscle, especially the Ca^{2+} sensitivity of force development and ATPase activity in vitro and in vivo [13–17].

Previous reports have suggested that there is a more consistent relationship between certain genetic mutations and clinical outcome, allowing for the classification of "benign" and "malignant" mutations [18–30]. For example, a favorable prognosis has been reported in patients with a Phe110Ile mutation of the *TNNT2* mutation (in 16 individuals of 6 Japanese families) [19, 20]. From these data, genetic analysis and determination of genotype were thought to be important for assisting with patient management.

However, these findings were based on limited experience; to date, only 30 different mutations have been identified in *TNNT2* [9–11, 18]. In addition to this genetic diversity, the phenotypic expression of these mutations varies considerably, ranging from asymptomatic individuals with a normal life expectancy to those with sudden cardiac death or the need for an early heart transplant. Clinical parameters such as the degree of LVH, the presence or absence of a left ventricular outflow tract gradient, and electrophysiology testing have not been predictive markers of poor prognosis [1–5]. More recently, it was reported that late gadolinium enhancement with cardiac magnetic resonance can be a predictive marker of the ventricular arrhythmia and poor prognosis in HCM [31].

In recent years, mutation-specific risk stratification was considered to be not possible, but genetic test-based risk stratification seemed to be clinically informative [32].

The *TNNT2* mutation has the third-ranked incidence of familial HCM, and the genotype–phenotype correlation of this gene still remains insufficient in Japanese familial HCM. Therefore, in the present study we focused on screening the *TNNT2* mutation.

Patients and methods

Subjects

We genetically evaluated 173 patients (101 men and 72 women; 0–79 years old, median age 20 years) who were clinically diagnosed with familial HCM.

Pediatric patients with HCM were recruited from Tokyo Women's Medical University. Written informed consent was obtained from all study subjects in accordance with the Declaration of Helsinki. If patients were younger than 16 years, informed consent was given by their guardians. We assessed each patient by taking their history and performing a physical examination, and reviewed their medical records. All assessments were done with the approval of the Ethics Committees of Tokyo Women's Medical University.

The diagnosis of HCM was determined through clinical evaluation, chest radiography, electrocardiography, echocardiography, and cardiac catheterization based on current international consensus criteria.

Diseases of the heart, hypertension, valvular heart disease, or metabolic disease attributable to HCM were excluded from this study by pediatric cardiologists.

Mutation screenings

Genomic DNA was prepared from peripheral blood lymphocytes or lymphoblast cell lines transformed by the Epstein–Barr virus, as described previously [33]. *TNNT2* coding regions and exon–intron boundaries, including regions approximately 30–100 bp upstream and downstream from the exons, were amplified from genomic DNA using primers, as described in previous reports [34, 35]. Genomic DNA (50 ng) was amplified through the use of primers designed from flanking intron sequences (Table 1).

Amplified products were purified using a MultiScreen polymerase chain reaction (PCR) plate (Millipore, Billerica, MA, USA) and directly sequenced using the ABI-PRISM BigDye-terminator cycle sequencing reaction kit and ABI 3130*xl* genetic analyzer (Applied Biosystems, Foster City, CA, USA). When a mutation was detected, we confirmed that it was not presenting 363 Japanese normal chromosomes by direct sequencing.

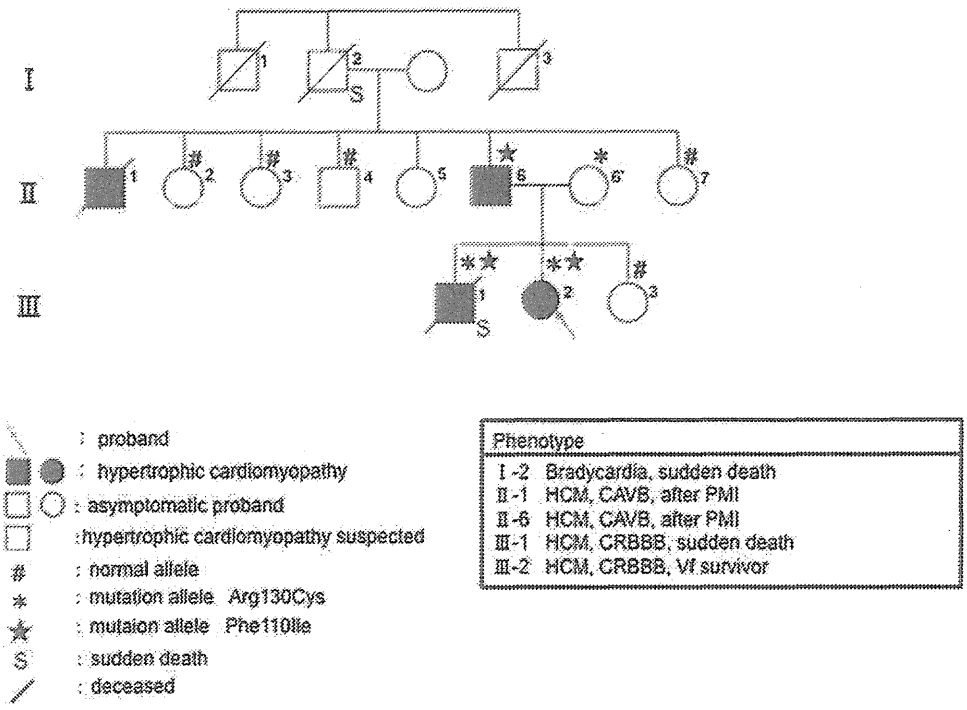

Fig. 1 Pedigree and phenotype of Family A. Pedigree with three generations (*Roman Numerals*), the propositus is denoted by an *arrow*. Further clinical data for family members is detailed in the *table*. *HCM* hypertrophic cardiomyopathy, *CAVB* complete atrioventricular block, *PMI* pacemaker implantation, *CRBBB* complete right bundle-branch block, *Vf* ventricular fibrillation

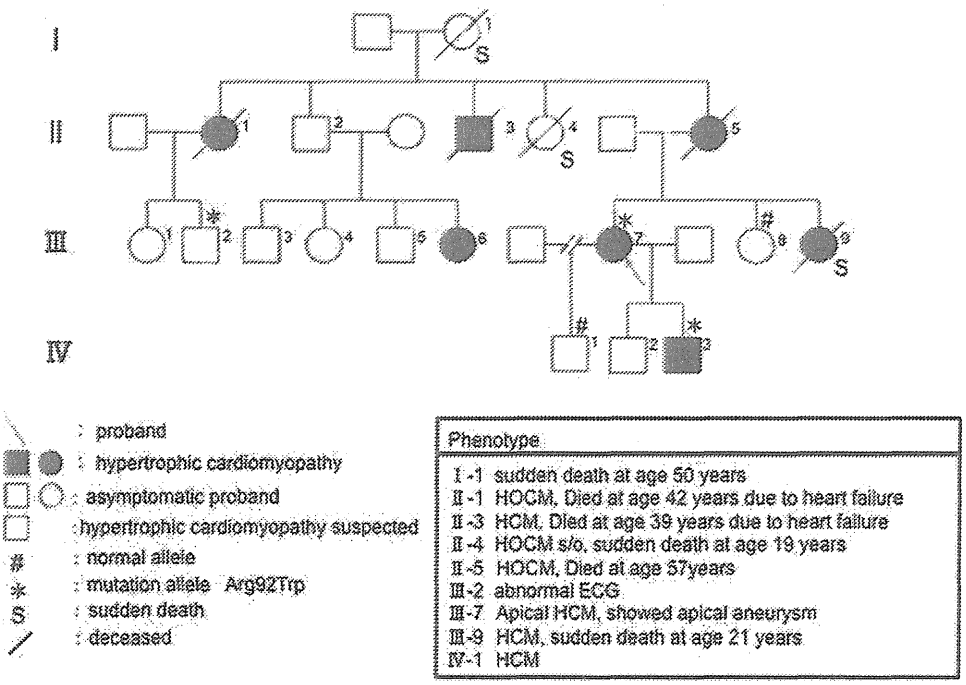

Fig. 2 Pedigree and phenotype of Family B. Pedigree with four generations (*Roman Numerals*), the propositus is denoted by an *arrow*. Further clinical data for family members is detailed in the *table*. *HCM* hypertrophic cardiomyopathy, *HOCM* hypertrophic obstructive cardiomyopathy, *ECG* electrocardiogram

brain damage, and was treated with corticotropin. DNA analysis showed that the proband (Fig. 3; IV-3) and her mother (Fig. 3; III-3) had the Arg92Trp mutation of *TNNT2*.

In family D, two of three members who carried or were suspected of having HCM died suddenly (Fig. 4; I-4, III-3), and one was a Vf survivor (Fig. 4; III-2). The proband (Fig. 4; III-2) had relatively strong cardiac hypertrophy (maximum wall thickness >20 mm), and cardiac standstill from ventricular fibrillation at the age of 24 years. Her cousin (Fig. 4; III-3) died suddenly at a young age (13 years), and her granduncle also died at the age of 40. DNA analysis shows that the proband had the 236T → C nucleotide alteration. This transition was observed in codon 79, and converted an isoleucine residue to a threonine residue (Fig. 5a). Ile79Thr occurred in conserved residues found in *TNNT2* orthologues of human, mouse, rat, cat, and

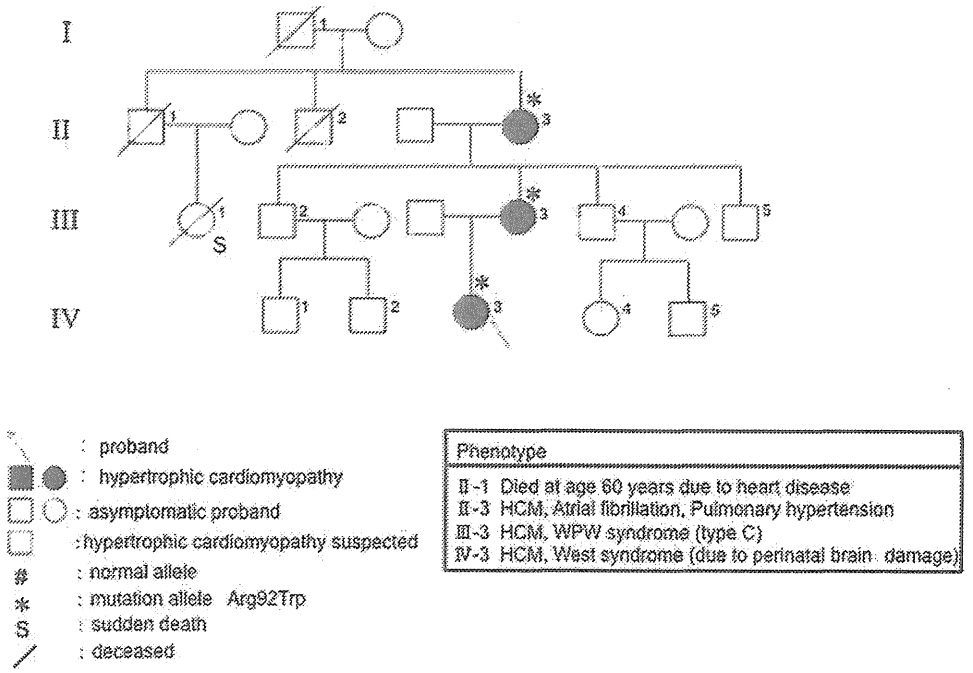

Fig. 3 Pedigree and phenotype of Family C. Pedigree with four generations (*Roman Numerals*), the propositus is denoted by an *arrow*. Further clinical data for family members is detailed in the *table*. *HCM* hypertrophic cardiomyopathy, *WPW syndrome* Wolff-Parkinson-White syndrome

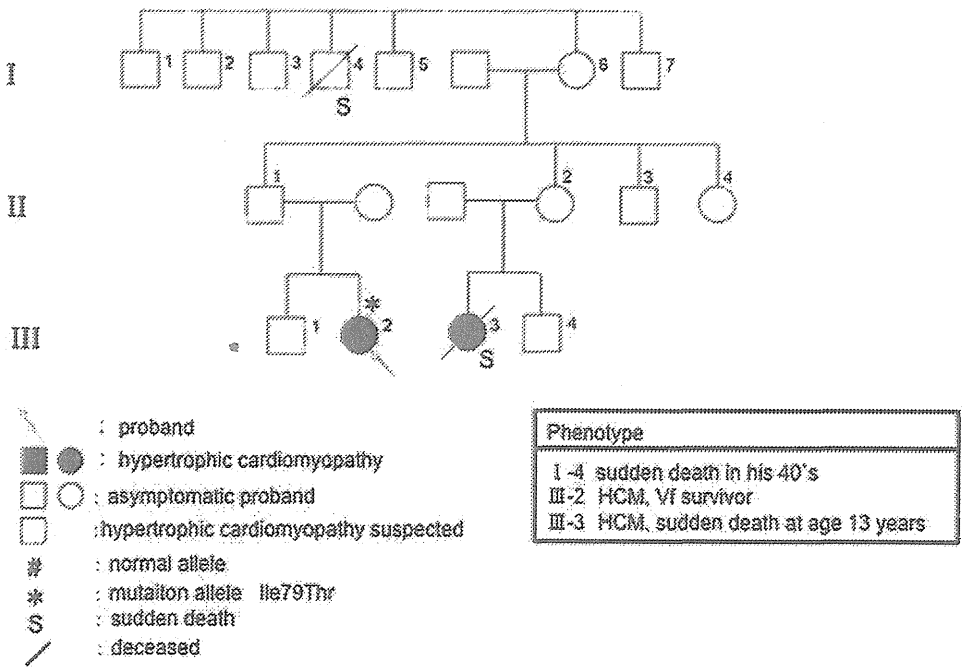

Fig. 4 Pedigree and phenotype of Family D. Pedigree with four generations (*Roman Numerals*), the propositus is denoted by an *arrow*. Further clinical data for family members is detailed in the *table*. *HCM* hypertrophic cardiomyopathy, *Vf* ventricular fibrillation

ox (Fig. 5b). This mutation was not observed in 363 chromosomes from unaffected Japanese populations. The proband (Fig. 4; III-2) also had a 5-bp (CTTCT) deletion/deletion polymorphism of intron 3 of *TNNT2* (Fig. 6a).

To clarify the clinical importance of this polymorphism, we performed genetic analysis in 47 HCM patients from 173 unrelated Japanese patients with familial HCM. In these 47 patients, 24 had the deletion/deletion polymorphism (51 %, 14 men and 10 women; 0–72 years old, median age 30 years old). Five of the 24 (21 %) patients who had the deletion/deletion polymorphism were diagnosed with apical HCM or HOCM. In 6 of the 10 (60 %) with available data of echocardiography among these 24 patients, the maximum wall thickness was more than 30 mm. On the other hand, in the 47 HCM patients, the remaining 23 did not have the deletion/deletion polymorphism, but had the deletion/insertion or insertion/insertion polymorphism (49 %, 15 men and 8 women; 0–76 years old, median age 18 years). Two of the 23 (9 %) patients were diagnosed with apical HCM, and there were no

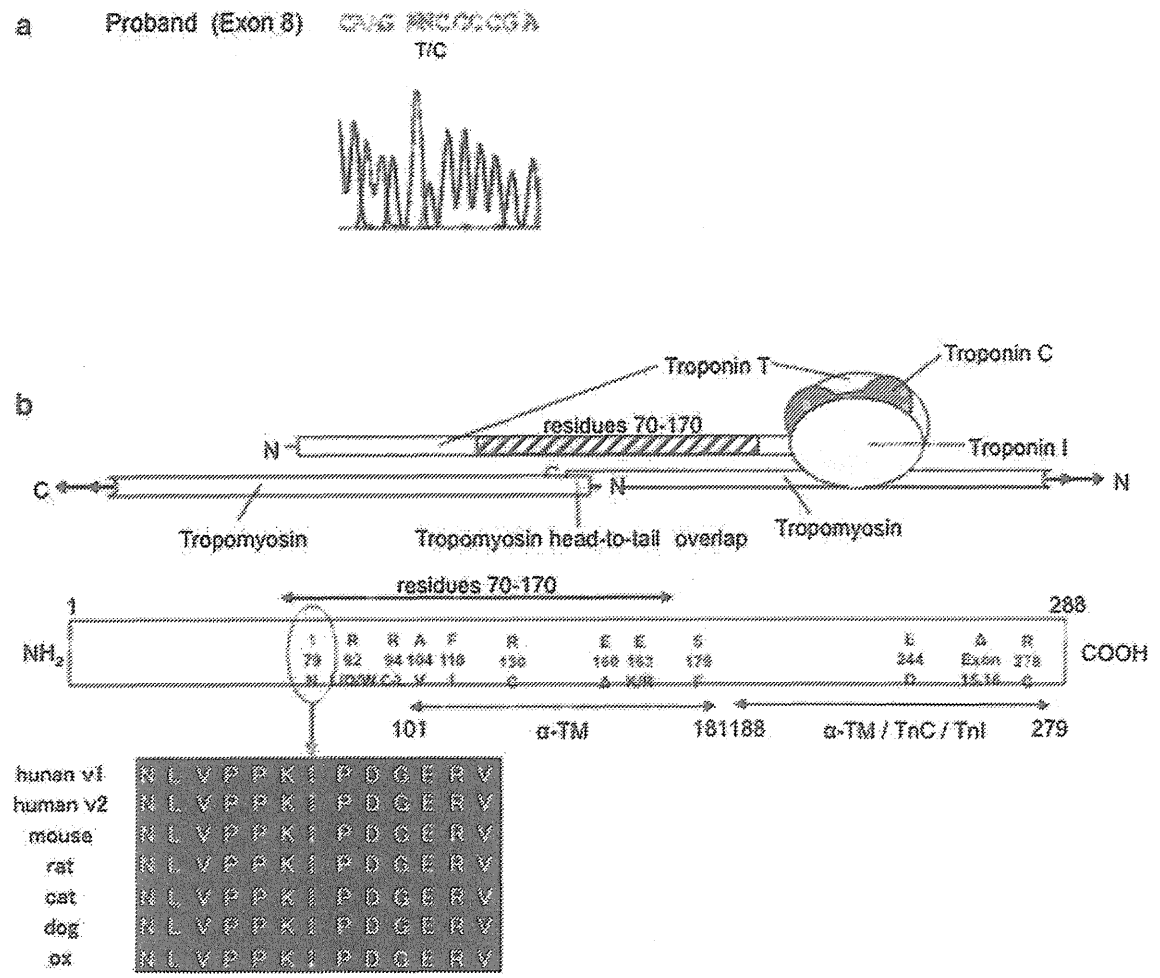

Fig. 5 a The new Ile79Thr mutation of *TNNT2* (*Exon 8*). b The position of the 79th Ile codes for the binding site of tropomyosin. This domain is well preserved in mammals

HOCM patients. Two of the seven (29 %) with available data of echocardiography among these 23 patients showed the maximum wall thickness of more than 30 mm.

Discussion

Previous studies have reported familial HCM cases where a consistent relationship between certain genetic mutations and clinical outcome was observed, allowing for the classification of "benign" and "malignant" mutations [18–30].

However, in recent years it was considered that the specific gene mutation cannot be the sole factor that dictates clinical phenotype. Since around 1 %–2 % of all patients with HCM had a "benign" or "malignant" mutation, mutation type was not seen to be clinically useful in predicting prognosis in HCM given this very low incidence rate [32, 38–40]. In addition, it was reported that mutation of a sarcomeric protein gene can cause RCM, HCM, and DCM within the same family, and patients with a benign mutation experienced a very serious clinical course [41, 42].

In the present study, the phenotype of each family was often different from reported cases, even if they had the same genetic mutation. In addition, families with the same genetic mutation showed a similar trend in the phenotype, but it was not exactly the same. However, sudden death in youth was observed in all of these families. On the other hand, there seemed to be a family with a "malignant" mutation, which did not show the phenotype of HCM or a family history of sudden death.

For example, in family A, two *TNNT2* mutations were found, the Arg130 Cys and Phe110Ile mutations. The Arg130 Cys mutation has been reported by a Chinese research group [21], whose clinical features had an early onset, syncope, sudden death in youth, heart failure, and arrhythmia as atrial fibrillation. The Phe110Ile mutation has been reported to show a favorable prognosis, and have comparatively slight hypertrophy or apical hypertrophy