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ARTICLE

High Prevalence of Genetic Alterations in Infantile-Onset Cardiomyopathy

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ABSTRACT

Background and Method: The genetic cause of infantile-onset cardiomyopathy is rarely investigated. Here, we conducted whole exome sequencing (WES) and mitochondrial DNA (mtDNA) sequencing in eight patients with infantile-onset cardiomyopathy to identify genetic variations. **Result:** Among these patients, two (25%) had dilated cardiomyopathy (DCMP), two (25%) had left ventricular non-compaction (LVNC), and four (50%) had hypertrophic cardiomyopathy (HCMP). Except four patients identified prenatally, the remaining patients presented at a median age of 85.5 days. WES identified genetic variants in a total of seven (87.5%) patients and mtDNA sequencing in the other case. *TPM1* and *MYH7* variants were identified in the two patients with DCMP; *MYH11* and *MYLK2* variants in the two patients with LVNC; *HRAS, BRAF*, and *MYH7* variants in three patients with HCMP; and *MT-ND1* variant in one patient with HCMP having high blood lactic acid levels. Among the eight variants, four were classified as pathogenic or likely-pathogenic according to the American College of Medical Genetics (ACMG) guidelines, and the remaining were identified as variants of unknown significance (VUSs). Three pathogenic mutations were *de novo*, whereas four (likely-pathogenic or VUSs) were inherited from a respective parent, excluding one variant where parental testing was unavailable, questioning whether these inherited variants are disease-causing. Three patients died before 3 months of age. **Conclusion:** Genomic studies, such as WES with additional mtDNA sequencing, can identify a genetic variant in high proportions of patients with infantile-onset cardiomyopathy. The clinical implication of the parentally inherited variant needs to be assessed in a larger patient and family cohort with a longitudinal follow-up.

KEYWORDS

Cardiomyopathy; whole exome sequencing; infantile-onset

1 Introduction

Cardiomyopathies are structural and functional heart muscle disorders that affect ventricular systolic function, diastolic function, or both. The incidence of cardiomyopathy is approximately one per 100,000 in children less than 18 years old [1,2]. Some studies have shown that cardiomyopathy below the age of one was four-to-eight times higher in incidence than that in the population over one year of age [1,3]. Cardiomyopathy in children has serious complications and high mortality rates despite the recent development of mechanical cardiopulmonary support and is the major causative disease of pediatric heart transplantation [4]. In particular, infantile cardiomyopathy that develops before 1 year of age, including congenital cardiomyopathy, has a worse prognosis than late-onset cardiomyopathy [5,6].

Generally, the most common cause of childhood cardiomyopathy is idiopathic [7]. However, recent developments in genetic testing have identified genetic alterations in a significant substantial number of previously classified idiopathic cardiomyopathies [8]. Compared to adults, pediatric patients have fewer comorbidities, such as arteriosclerosis and diabetes, and have relatively stronger associations with genetic alterations. Indeed, several studies have been conducted to identify genetic causes in pediatric patients with cardiomyopathy [9,10].

As more than 60 genes are associated with cardiomyopathy, multi-gene testing using next-generation sequencing techniques, such as panel-gene testing, is an appropriate diagnostic method. However, panel-gene testing cannot identify genetic causes outside the list of genes in the designed panel. Moreover, the time, cost, and effectiveness of a genomic study must be considered; whole exome sequencing (WES) is considered one of the fastest diagnostic tools in cardiomyopathy [4,11]. However, to our knowledge, no studies have been conducted to characterize infantile-onset cardiomyopathy with high mortality rates and genetic background.

Therefore, the aim of the present study was to identify variants by performing WES and additional mitochondrial DNA (mtDNA) sequencing in patients with infantile-onset cardiomyopathy. By assessing the pathogenicity of the variant and its existence in the parents, we investigated the association of the identified variant with the disease in detail.

2 Material and Methods

2.1 Subjects and Clinical Evaluation

WES was conducted in patients with cardiomyopathy diagnosed at Asan Medical Center Children's Hospital, Seoul, Korea between August 2018 and June 2020. Among them, eight patients, including four with congenital cardiomyopathy suspected at prenatal examination, were infantile-onset and included in this study. Detailed demographics and clinical characteristics of the patients were reviewed, including age, initial presentations, gender, family history, laboratory results, radiological results, and genetic test results.

Informed consent was obtained from the parents of the patients for the genetic test. This study was approved by the Institutional Review Board for Human Research of Asan Medical Center (IRB numbers 2018-0574 and 2018-0180).

2.2 Definitions of Cardiomyopathies

- Dilated cardiomyopathy (DCMP): Left ventricular fractional shortening (FS) or ejection fraction (EF) > 2 SD below the normal mean for age and Left ventricular end-diastolic dimension (LVEDD) or volume > 2 SD above the normal mean for body surface area [4,12].
- Left ventricular non-compaction (LVNC): Prominent LV trabeculations with deep recesses communicating with the LV cavity and a thin, compacted epicardial layer [13].
- Hypertrophic cardiomyopathy (HCMP): Left ventricular posterior wall thickness at end diastole >2 SD above the normal mean for body surface area [14].

2.3 Variant Annotation and Interpretation by WES Analysis

WES was performed using genomic DNA isolated from patient's whole blood or a buccal swab sample. All exons of all human genes (approximately 22,000) were captured using a Twist Human Core Exome Kit (Twist Bioscience, San Francisco, CA, USA). The captured genomic regions were sequenced using a NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Raw genome sequencing data analysis included alignment to the reference sequence (NCBI genome assembly GRCh37; accessed February 2009). The mean depth of coverage was 100-fold with 99.2% higher coverage than 10-fold. The pathogenicity of each variant according to the American College of Medical Genetics (ACMG) guidelines [15] and relevant patient phenotypes were assessed using the automated variant interpretation system EVIDENCE [16]. Candidate variants based on EVIDENCE were reviewed and selected by medical geneticists. Sanger sequencing of the variant identified via exome sequencing was performed for patients and their parents.

2.4 Whole Mitochondrial DNA Sequencing

Genomic DNA was isolated from the peripheral blood using a PUREGENE DNA isolation kit (Gentra, Minneapolis, MN, USA). The amplifications by PCR were performed in 30 cycles. After verifying that the single-specific PCR product was amplified, DNA sequencing was performed using the same primers used in PCR and BigDye Terminatore V3.1 Cycle Sequencing Ready reaction kit (Applied Biosystems, Foster city, CA, USA) according to the manufacturer's instructions. Electrophoresis and analysis of the reaction mixtures were conducted with an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

3 Results

The patient's clinical data are summarized in Tab. 1. There were three male and five female patients. Two of eight patients (25%) had DCMP (patient Nos. 1, 2), two (25%) had LVNC (patient Nos. 3, 4), four (50%) had HCMP (patient Nos. 5–8). Four patients were suspected as cardiomyopathy prenatally, and the other four were identified at 85.5 [interquartile range (IQR) 73.5–138.3] days of life.

Table 1: Clinical data of patients with infantile-onset cardiomyopathy

Pt ID	Type of CMP	Sex	Onset age (day)	Initial presentation	Admission duration (days)	Coexisting symptom	Family history	EF (%)	FS (%)	LV mass index (g/m^2)	LVOTO	Mechanical circulatory support devices	Mortality
1	DCMP	F	77	Poor oral intake	28	–	–	32	15	103	–	LVAD	NA
2	DCMP	M	0	Prenatal	58	–	Paternal cousin with myocarditis	21	9	95	–	–	–
3	LVNC	F	0	Prenatal	7	Large VSD	Paternal AR	22	9	75	–	–	Expired
4	LVNC	F	63	Murmur	11	–	-	22	10	119	–	–	–
5	HCMP	M	0	Prenatal	48	Dysmorphic face, polydactyly, cystic hygroma	–	93	62	22	o	–	Expired
6	HCMP	M	271	Murmur	0	Dysmorphic face	–	86	53	71	o	–	–
7	HCMP	F	0	Prenatal	87	TGA, VSD, CoA	–	26	11	150	o	ECMO	Expired
8	HCMP	F	94	Lethargy	52	Stroke, lactic acidosis	–	90	58	170	o	–	–

3.1 Clinical Presentation

The presenting signs in those diagnosed later were heart murmur (two patients), lethargy (one patient), and poor oral intake (one patient). All patients were hospitalized in the intensive care unit, and their median duration of admission was 48 (IQR 19.5–55) days. Two patients (25%) were provided mechanical circulatory support; three (37.5%) died at 48 [27.5–67.5] days of life.

Five patients had coexisting symptoms. Structural abnormalities in the heart were found in two patients—patient No. 3 had a large ventricular septal defect (VSD) with a diameter of 12 mm and patient No. 7 had transposition of great arteries, VSD, and coarctation of aorta. Multiple extracardiac anomalies with dysmorphic face were found in two patients—patient No. 5 had macrocephaly, cystic hygroma, epicanthal fold, proptosis, low set ears, micrognathia, widely spaced nipples, and undescended left testis and patient No. 6 had fragile hair, low set ears, and a webbed neck. Patient No. 8 showed symptoms of metabolic diseases, such as hemorrhagic stroke and severe lactic acidosis [>15 mmol/L (ref. 0.56–1.390)]. The median (IQR, reference range) of EF and FS for the two patients with DCMP was 27% (24–29%, ref. > 50) and 12.5% (11–14%, ref. > 28), respectively; the median (IQR, reference range) LV mass index for four patients with HCMP was 110 g/m^2 (59–155 g/m^2, ref. >95), and all four had a degree of left ventricular outflow tract obstruction (LVOTO).

The echocardiography images representing each phenotype are summarized in Fig. 1. Patient No. 1 with DCMP shows dilated left ventricle (Figs. 1A, 1B), Patient No. 4 with LVNC shows non-compacted excessive trabeculation (Figs. 1C, 1D), Patient No. 7 with HCMP shows severe thickening of the ventricular wall (Figs. 1E, 1F). The electrocardiogram representing each phenotype is shown in Fig. 2. Left ventricular hypertrophy was observed in patient No. 1 with DCMP and patient No. 4 with LVNC (Figs. 2A, 2B), and biventricular hypertrophy and wall strain pattern were observed in patient No. 7 with HCMP (Fig. 2C).

3.2 Genetic Variants

WES identified a genetic variation in seven out of eight patients. A detailed analysis of the genetic variation is summarized in Fig. 3 and Tab. 2. WES was carried out, and common variants corresponding to 98% with a minor allele frequency >5% were filtered out. Only variants matching the disease reported to date were screened and, among them, likely-benign, benign, and non-coding variants with low evidence according to the ACMG guidelines [15] were excluded. Lastly, the final candidate genetic variant was selected by a medical geneticist considering the relationship between gene and patient phenotypes.

In the two patients with DCMP, genetic variations were identified in the genes *TPM1*, which is responsible for DCMP1Y (OMIM 611878, www.omim.org), and *MYH7*, which is responsible for DCMP1S (OMIM 160760). According to the ACMG guidelines [15], two variants, c.142G > T (p. Asp48Tyr) in *TPM1* and c.2775G > T (p. Arg925Ser) in *MYH7*, were classified as variants of unknown significance (VUSs). Parental tests were available in patient 2, which was inherited from an asymptomatic parent.

In the two patients with LVNC, the *MYH11* variant for aortic aneurysm, familial thoracic 4 (AAT4) (OMIM 160745) and the *MYLK2* variant for cardiomyopathy, hypertrophic 1 (CMH1) (OMIM 606566) were identified. According to the ACMG guidelines [15], two variants, c.5260G > C (p. Glu1754Gln) in *MYH11* and c.1577 + 1G > A in *MYLK2*, were classified as VUSs. All mutations were inherited from one parent; the father of patient No. 3 had aortic regurgitation, and the parent of patient No. 4 was asymptomatic.

In the four patients with HCMP, the genetic variations were identified in the genes *HRAS*, which is responsible for Costello syndrome (OMIM 190020), *BRAF*, responsible for Cardiofaciocutaneous syndrome (CFC, OMIM 164757), and *MYH7*, responsible for cardiomyopathy, hypertrophic 1 CMH1 (OMIM 160760). According to the ACMG guidelines [15], one variant, c.1182C > A (p. Asp394Glu) in

MYH7 was classified as likely-pathogenic and the other two variants, c.35G > A (p. Gly12Asp) in *HRAS* and c.1502A > T (p. Glu501Val) in *BRAF*, were classified as pathogenic. Parental tests were available for the three patients; the *HRAS* and *BRAF* variants were *de novo*, and the *MYH7* variant was inherited from an asymptotic parent. However, WES did not identify a mutation in patient No. 8, but mtDNA sequencing found a pathogenic mutation, m.3946G > A (p. Glu214Lys), in *MT-ND1*, which has been reported in patients with mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes (MELAS; OMIM 540000). No relevant mutation was found in the mother's mtDNA sequencing.

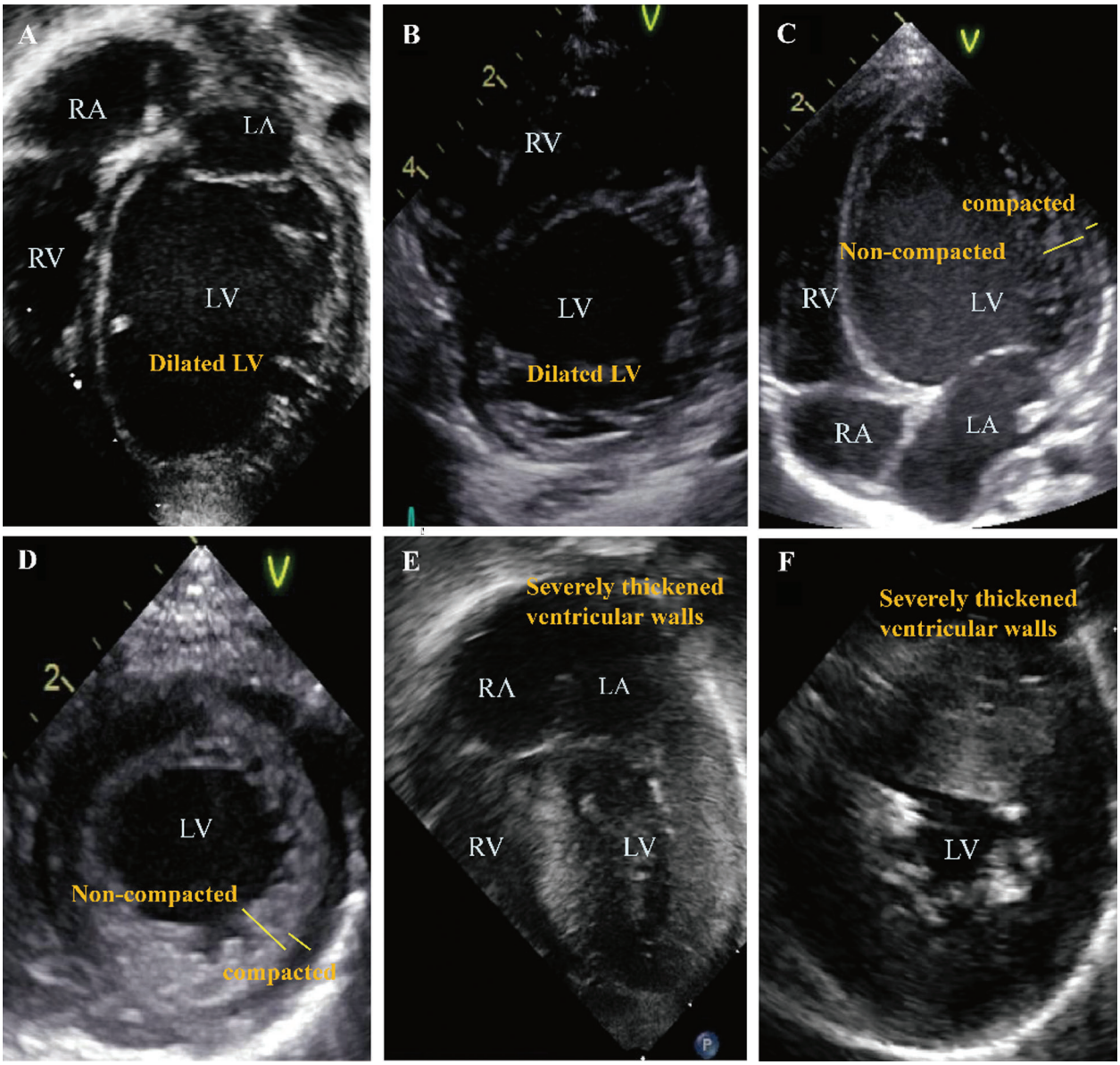

Figure 1: Echocardiographic 4-chamber and parasternal short axis images for different phenotypes. (A, B) An infant with a dilated cardiomyopathy (patient No. 1), (C, D) A neonate with a left ventricular non-compaction cardiomyopathy (patient No. 4), (E, F) A neonate with a hypertrophic cardiomyopathy (patient No. 7). Abbreviations: RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle

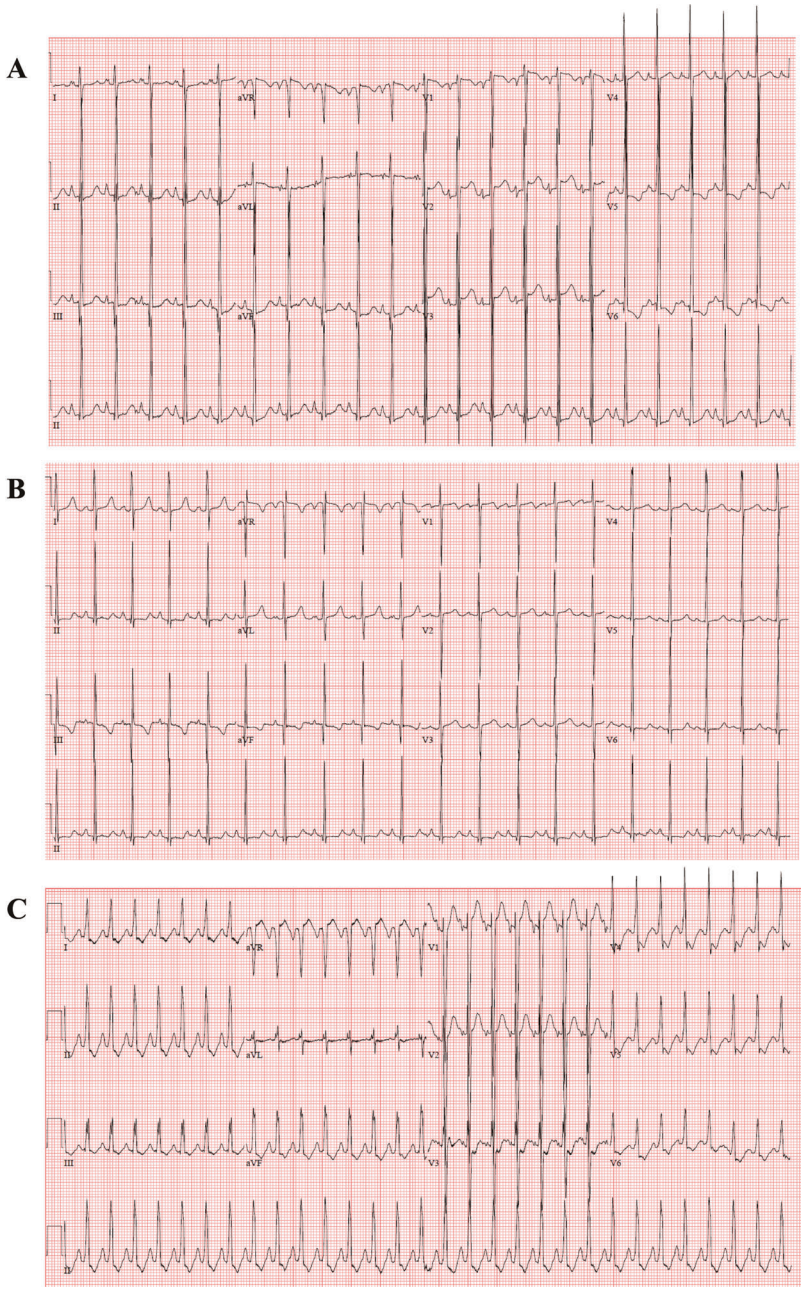

Figure 2: Electrocardiogram for different phenotypes. (A, B) An infant with a dilated cardiomyopathy (patient No. 1), (C, D) A neonate with a left ventricular non-compaction cardiomyopathy (patient No. 4), (E, F) A neonate with a hypertrophic cardiomyopathy (patient No. 7)

3.3 Clinical Course

The clinical courses of the subjects are summarized in Tab. 3. Patient No. 1 was lost to follow-up after 1 month of life because of distance contraint from home. Among the seven patients with a follow-up period [median (IQR), 6 (1–10) months], one (12.5%, patient No. 4) is recovering heart function, three (37.5%, patient Nos. 2, 6, and 8) have stationary heart function with medications, and three (37.5%, patient Nos.

3, 5, and 7) had died; these were all identified prenatally. Median (IQR) survival duration was 48 (27.5–67.5) days of life (Fig. 4).

Table 2: Genetic variations found in patients with infantile-onset cardiomyopathy

Pt ID	Gene name	Position	Nucleotide change	Protein change	GenBank number	Predicted pathogenicity*	Bayesian probability	Genetic origin	*In silico*†
1	*TPM1*	chr15:63349193	**c.142G > T**	**p.Asp48Tyr**	NM_001330351.1	VUS	0.812	-#	REVEL: 0.84
2	*MYH7*	chr14:23893263	**c.2775G > T**	**p.Arg925Ser**	NM_000257.3	VUS	0.675	Paternal	REVEL: 0.83
3	*MYH11*	chr16:15812228	**c.5260G > C**	**p.Glu1754Gln**	NM_001040114.1	VUS	0.812	Paternal	REVEL: 0.82
4	*MYLK2*	chr20:30419659	**c.1577 + 1G > A**		NM_033118.3	VUS	0.499	Maternal	ADA: 0.99, RF: 0.94
5	*HRAS*	chr11:534288	c.35G > A	p.Gly12Asp	NM_001130442.2	Pathogenic	0.999	*De novo*	REVEL: 0.78
6	*BRAF*	chr7:140477806	c.1502A > T	p.Glu501Val	NM_004333.4	Pathogenic	0.999	*De novo*	REVEL: 0.99
7	*MYH7*	chr14:23898513	c.1182C > A	p.Asp394Glu	NM_000257.3	Likely pathogenic	0.949	Paternal	REVEL: 0.59
8	*MTND1*		m.3946G > A	p.Glu214Lys	NC_012920.1	Pathogenic		*De novo*	

Notes: #Parent test was not performed
*As presented in the consensus statement of the ACMG (American College of Medical Genetics) [15]
†*In silico* prediction: Rare exome variant ensemble learner (REVEL), adaptive boosting (AdaBoost), random forest (RF) score [17,18]
Bold: novel variant

Among the two patients with DCMP, patient No. 1 was lost from follow-up and patient No. 2 had a persistently low cardiac output of 31% in EF but had normal growth and development until 6 months of age.

Among the two patients with LVNC, patient No. 3 died 1 week after birth due to progressive heart failure, and patient No. 4 is now recovering heart function to EF 47%, showing normal development and growth until 35 months of age.

Among the four patients with HCMP, patient No. 5 with Costello syndrome had progressive LVOTO and died due to cardiogenic shock at 48 days of life and patient No. 7 had an open-heart operation and died from failure of extracorporeal membrane oxygenation weaning at 86 days of life. Patient No. 6 with CFC has a similar level of hypertrophy and LVOTO until 13 months of age and is currently taking an anti-epileptic drug and undergoing rehabilitation for epilepsy and global development delay (case 17 in a previous study by Lee et al. [19]). Patient No. 8 with MELAS is being treated with vasodilator, diuretics, and anti-epileptic drug for seizure. At 7 months of age, she was below the third percentile in height and weight. She could make eye contact but could not control her head. Cardiac hypertrophy and LVOTO were stationary, but the blood lactic acid levels could be decreased to 3.7 mmol/L upon treatment with multivitamins, coenzymes, and arginine.

4 Discussion

In this study, all patients with infantile-onset cardiomyopathy were identified based on variants associated with cardiomyopathy via WES and additional mtDNA sequencing. Kindel et al. [8] identified genetic etiology in 76% of pediatric patients with cardiomyopathy, and a comparable or somewhat higher frequency was found in our study. Moreover, in our study, all the genetic variants found in HCMP were pathogenic or likely-pathogenic, whereas the variants in DCMP or LVNC were VUSs. It has been the general consensus that genetic alteration is a major contributing factor to the development of infantile-onset cardiomyopathy. Pediatric patients with cardiomyopathy generally survive up to a median age of

three, and the 1year rate of death is 31% [3]. The higher mortality and earlier death age observed in the present study [3/8 (37.5%), 48 (27.5–67.5) days] indicate that infantile-onset cardiomyopathy may hold a severe clinical course, which is attributed to the genetic factors.

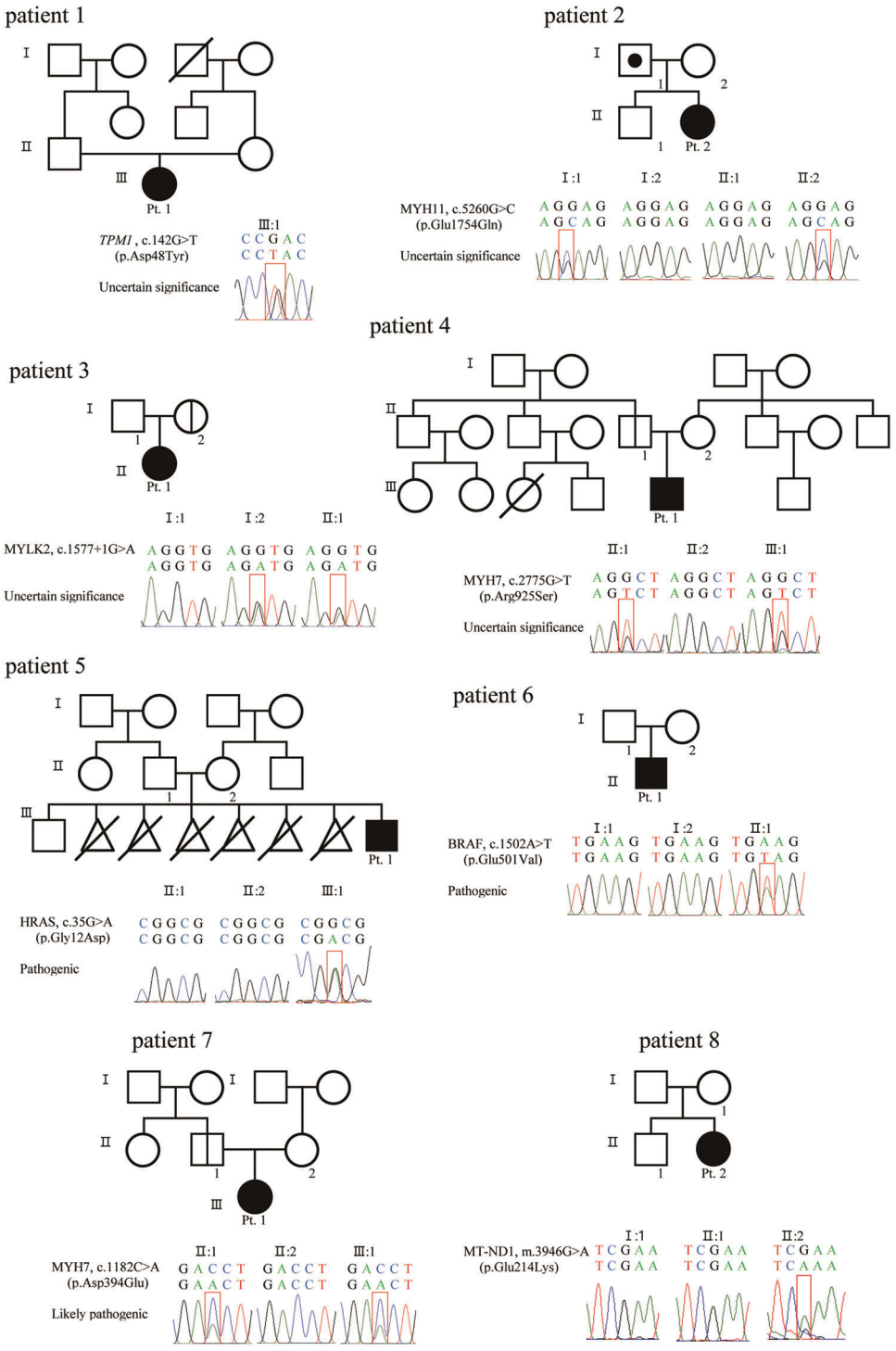

Figure 3: Sanger sequence of variant and pedigree for patients and parents

Table 3: Clinical courses of patients with infantile-onset cardiomyopathy

Pt ID	Current age (10/2020)	Height	Weight	Development	Cardiovascular medication	Cardiovascular disease course	Other medical conditions
1	HF managed for 28 days (including 20 days of ICU stay) then discharged and lost to follow-up						
2	6 m	75–85 p	10–25 p	Normal	1 vasodilator, 3 diuretics, 1 inotropic	EF 31%, RV function recovered and considering reducing LV loading by PAB	–
3	HF aggravation caused cardiogenic shock and death despite maximum dose of inotropic at 7 days old						
4	2 y 11 m	90–95 p	50–75 p	Normal	2 vasodilators, 1 diuretic	EF recovered up to 47%	–
5	LVH progressed rapidly and the patient failed to recover from cardiogenic shock and LVOTO and died at 48 days old						
6	1 y 1 m	50–75 p	95–97 p	Global developmental delay	1 vasodilator	Stationary	AED with infantile spasms at 1 year old, keeping stationary supravalvar PS 2.3 m/s
7	Thirteen days after birth, arch repair, atrial septectomy, PAB, and PDA division were conducted, but ECMO weaning failure and cardiogenic shock was irreversible; the patient died at 87 days old						
8	7 m	<3 p	<3 p	Global developmental delay	1 vasodilator, 3 diuretics	Stationary	After diagnosis of MELAS, taking AED with multivitamins and coenzyme therapy

Abbreviations: EF, ejection fraction; HF, heart failure; ICU, intensive care unit; MVR, mitral valve replacement; RV, right ventricle; LV, left ventricle; PAB, pulmonary artery banding; LVH, left ventricular hypertrophy; LVOTO, left ventricle outflow tract obstruction; AED, anti-epileptic drug; PS, pulmonary stenosis; PDA, patent ductus arteriosus; ECMO, extracorporeal membrane oxygenation; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes

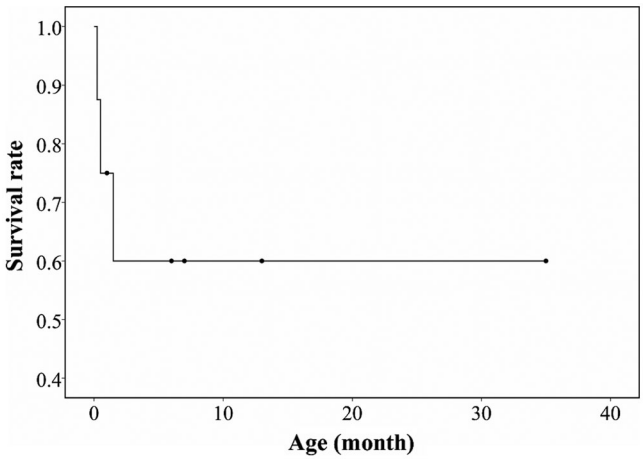

Figure 4: Kaplan-Meier survival graph of the patients with infantile-onset cardiomyopathy

In general, the phenotype suggested by a genetic defect identified in each of the patients was consistent with that of the affected patient. Genetic variants in patients with DCMP and HCMP in this study have been found in similar phenotypes in previous studies [9,20]. However, genetic variants found in patients with LVNC (*MYH11* and *MYLK2)* have not been reported as being directly related to the disease phenotype. The *MYLK2* variants have been previously described as being associated with HCMP [21]. Meanwhile, in a previous study, the *MYLK2* variant was associated with LVNC, as with our patient No. 4 [22]. The *MYH11* variant in patient No. 3 was associated with the development of aortic aneurysm, patent ductus

arteriosus, or HCMP [23]. During the human embryonic stage, the development of the coronary artery is important for the disappearance of the sinusoids and transformation of the spongy myocardium into compact musculature. Therefore, it is possible that the *MYH11* variant disturbs this transformation of fetal cardiac musculature by altering the myosin heavy-chain formation in the fetal blood vessels [24,25]. LVNCs might be considered a distinct subtype in the spectrum of cardiomyopathy but is still classified as a sub-trait or unclassified cardiomyopathy, given that LVNCs are found in other types of cardiomyopathy (DCMP or HCMP) [26,27]. As most patients have LVNC with other cardiomyopathies rather than as an isolated phenotype, the causative association of a gene to the development of LVNC is not yet clear [22,28]. Therefore, further experience with more cases of isolated LVNC or LVNC without DCMP or HCMP is required to assess this causative association.

In this study, one patient (patient No. 8) was diagnosed with MELAS through whole mtDNA sequencing, although WES did not find pathogenic variants in her nuclear DNA. Cardiomyopathy is one of the most characteristic features of mitochondrial disease, and her high blood lactic acid level was an additional pathognomonic finding to suspect a mitochondrial disease [20,29]. Indeed, infantile cardiomyopathy can be the first manifestation in some patients with mitochondrial disease [30]. Therefore, mtDNA sequencing should be considered in addition to or even before WES in patients with infantile-onset cardiomyopathy and high blood lactic acid level.

Importantly, one of the greatest challenges in the interpretation of genetic testing results in cardiomyopathy is that many parents share the variant found in their affected child but they do not have any subjective, relevant symptoms [31,32]. In this study, the parents carrying the same variant of their respective child were mostly asymptomatic (3/4, 75%). As most of the hereditary cardiomyopathies are inherited in an autosomal-dominant manner, a varying degree of penetrance and expressivity is expected, which can be affected by genomic, epigenetic, or environmental factors [33]. Not only RNA content and DNA sequence but also methylation status, chromatin structure and accessibility, protein composition, cell history, microenvironment, and cellular states *in situ* can affect the phenotype of subcellular, cellular, and tissue scales and finally that of the system scales [34,35]. In particular, *TPM1, MYLK2*, and *MYH7* variants, found in the asymptomatic parents, were reported to confer a variable degree of penetrance and expressivity [36–38].

As subclinical cardiomyopathy is likely to progress gradually, a single evaluation is not sufficient to determine whether the asymptomatic carrier is not affected by cardiomyopathy; thus, the identified variant is not the causative variant. Rather, assessing their cardiac function on a regular basis for the long-term should be considered. There are no defined guidelines for these genotype-positive/phenotype-negative patients. However, those parameters would be helpful to predict the cardiomyopathy in the early stage, such as left ventricular global longitudinal strain, peak left atrial longitudinal strain that can be measured by cardiac magnetic resonance imaging, or three-dimensional echocardiography [39–43].

As an increasing number of subjects become involved in genomic studies such as WES, a major concern is the interpretation of variants identified in each subject. Importantly, according to the ACMG guidelines, the variants in the genes associated with cardiomyopathy should be considered to inform the subject regardless of the primary medical condition for which the genomic study was required [44]. In this respect, prediction for the pathogenicity of each identified variant is very important to interpret the causality of each variant. The population frequency, location of a variant, characteristics of a variant (frameshift, splicing, nonsense, and missense) and *in silico* analysis tools, such as REVEL, ada, and rf score, have been used to determine pathogenicity in accordance with ACMG guidelines (pathogenic, likely-pathogenic, VUS, likely-benign, and benign) [15–18,45,46]. In this study, only two of seven nuclear DNA variants, classified as pathogenic according to the ACMG guidelines, were *de novo*, whereas all the likely-pathogenic variants or VUSs were found in either parent. Among them, only two families with VUSs have relevant clinical

symptoms or a family history. Therefore, the question remains that these VUSs might not be responsible for the development of CMP, and how much importance a physician should place on the variant, especially VUS, during genetic counseling for the family with the affected patient. In particular, as most parents are of reproductive age, whether to perform prenatal genetic testing for the next pregnancy based on the variant identified in the affected patient must be cautiously approached. To obtain a more relevant solution, larger scale genomic data with longitudinal clinical data of the asymptomatic but variant-carrying family members are required.

There are several limitations to this study. Due to the small number of patients, it was impossible to perform subgroup analysis among the patients with infantile cardiomyopathy. In addition, as mentioned earlier, the interpretation of the pathogenicity of each variant was limited by the observation of the same variant in asymptomatic family members. Due to the variable and mostly short-term follow-up periods, it was difficult to describe the long-term outcome of infantile cardiomyopathy in association with the genetic findings, which must be re-evaluated in a larger cohort with longer-term evaluation. Lastly, multiple variants in different genes can be found in a single patient [47], but in our study, only a single genetic variant was filtered as responsible for the patient phenotype. As we applied the artificial intelligence-based pipeline for the evaluation of genome data [16], the criteria for the filtration might have missed some low impact variants, which would have contributed to the development of cardiomyopathy together with the variant identified in each patient.

5 Conclusion

Genomic studies, such as WES, can identify a genetic variant in high proportions of patients with infantile-onset cardiomyopathy. However, due to a wide range of penetrance and expressivity, the interpretation of each variant's pathogenicity is limited in most cases.

Data Sharing: Not applicable.

Author Contribution: JP: Drafted and revised the manuscript critically for important intellectual content and substantially contributed to the interpretation of data. GHS, MK: Substantially contributed to the analysis and interpretation of data. YL, YC, J-KK, Y-HK, JJY, EA-RK, EJ, BSL: Substantially contributed to the acquisition of data. JSB, BHL: Designed the study, revised the manuscript critically for important intellectual content, and approved the final version to be published.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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