



Article

Left Ventricular Noncompaction and Congenital Heart Disease Increases the Risk of Congestive Heart Failure

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Abstract: Background: Left ventricular noncompaction (LVNC) is a hereditary cardiomyopathy that is associated with high morbidity and mortality rates. Recently, LVNC was classified into several phenotypes including congenital heart disease (CHD). However, although LVNC and CHD are frequently observed, the role and clinical significance of genetics in these cardiomyopathies has not been fully evaluated. Therefore, we aimed to evaluate the impact on the perioperative outcomes of children with concomitant LVNC and CHD using next-generation sequencing (NGS). Methods: From May 2000 to August 2018, 53 Japanese probands with LVNC (25 males and 28 females) were enrolled and we screened 182 cardiomyopathy-associated genes in these patients using NGS. Results: The age at diagnosis of the enrolled patients ranged from 0 to 14 years (median: 0.3 months). A total of 23 patients (43.4%) were diagnosed with heart failure, 14 with heart murmur (26.4%), and 6 with cyanosis (11.3%). During the observation period, 31 patients (58.5%) experienced heart failure and 13 (24.5%) developed arrhythmias such as ventricular tachycardia, supraventricular tachycardia, and atrioventricular block. Moreover, 29 patients (54.7%) had ventricular septal defects (VSDs), 17 (32.1%) had atrial septal defects, 10 had patent ductus arteriosus (PDA), and 7 (13.2%) had Ebstein's anomaly and double outlet right ventricle. Among the included patients, 30 underwent surgery, 19 underwent biventricular repair, and 2 underwent pulmonary artery banding, bilateral pulmonary artery banding, and PDA ligation. Overall, 30 genetic variants were identified in 28 patients with LVNC and CHD. Eight variants were detected in *MYH7* and two in *TPM1*. Echocardiography showed lower ejection fractions and more thickened trabeculations in the left ventricle in patients with LVNC and CHD than in age-matched patients with VSDs. During follow-up, 4 patients died and the condition of 8 worsened postoperatively. The multivariable proportional hazards model showed that heart failure, LV ejection fraction of < 24%, LV end-diastolic diameter z-score of > 8.56, and noncompacted-to-compacted ratio of the left ventricular apex of > 8.33 at the last visit were risk factors for survival. Conclusions: LVNC and CHD are frequently associated with genetic abnormalities. Knowledge of the association between CHD and LVNC is important for the awareness of clinical implications during the preoperative and postoperative periods to identify the populations who are at an increased risk of additional morbidity.

Keywords: left ventricular noncompaction; congenital heart disease; congestive heart failure; non-ischemic cardiomyopathy; genetics

1. Introduction

Left ventricular noncompaction (LVNC) is the most recently classified cardiomyopathy. First described in 1990, it is characterized by a pattern of thickened trabeculations and deep intertrabecular fossa communicating with the left ventricular (LV) cavity [1]. LVNC has a wide spectrum, ranging from asymptomatic to severe congestive heart failure (CHF) with concomitant risks of arrhythmia, systemic thromboembolization, and sudden cardiac death. Although its diagnosis has primarily focused on the identification of trabeculation, other features are important to classify the specific subtypes of LVNC [2]. The specific phenotype of LVNC has the risk of adverse clinical outcomes in children and may occasionally be seen in association with congenital heart disease (CHD) [3].

The etiology of LVNC in patients with CHD has been unknown [4]. Previously, LVNC occurs most frequently in patients with Ebstein's anomaly, following with septal defects, LV outflow tract obstructive lesions, hypoplastic left heart syndrome, and other right heart lesions [3–5]. However, the natural history of patients with LVNC and CHD has not been fully elucidated. In addition, it is considered difficult to estimate prognosis and to identify the surgical indications in children with LVNC because they have highly variable clinical presentations. Therefore, the aim of our study was to evaluate the impact on the perioperative outcomes of patients with concomitant LVNC and CHD.

2. Methods

2.1. Subjects

From May 2000 to August 2018, 53 Japanese probands with LVNC and CHD were referred to our institution for genetic testing from several institutions in Japan. Patients aged < 18 years diagnosed as LVNC at the participating institutions were eligible for this study. Patients with secondary etiologies of cardiomyopathy (e.g., endocrine, rheumatic, pulmonary, and immunologic diseases; systemic hypertension; and cardiotoxic exposures), those with a pacemaker because of rhythm disturbance, and those with non-follow-up records were excluded. Clinical data were retrospectively retrieved from the patients' medical records according to the following time course: initial visit, preoperation, postoperation, and last visit. Cardiac death, LV assist device implantation, heart transplant (HT), and implantable cardioverter-defibrillator (ICD) shock were classified as major adverse cardiac events (MACEs).

Age matched patients with ventricular septal defects (VSDs) were selected from the Toyama University Hospital Database for comparison. All these patients underwent a surgery during the same period to endorse similarity of medical management.

Informed consent was gained from all patients or their guardians according to the institutional guidelines. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the *a priori* approval of the Research Ethics Committee of the University of Toyama, Japan.

2.2. Endpoint Assessment

Primary outcome was the time to the combined endpoint of MACEs, whereas secondary outcomes were arrhythmia, thromboembolic events, echocardiographic parameters, and genetic status.

2.3. Electrocardiogram Collection

All electrocardiograms were assessed independently by two well-trained investigators (K.H. and N.M.) who were blinded to the clinical data. The two investigators judged more than 95% consistency. The final judgment was made by a third experienced investigator (electrophysiologist) in

cases of disagreement. The criteria for J wave and fragmented QRS were based on the description by Antzelevitch, Yan, and Das [6,7].

2.4. Echocardiographic Data Collection

Echocardiography (two-dimensional, color Doppler, and M-mode) was performed to assess cardiac structure, ventricular size and function (fractional shortening and ejection fraction (EF)), and valvular regurgitation. The diagnosis of congestive heart failure (CHF) was defined by clinical findings of tachypnea, feeding difficulty, and cyanosis; cardiomegaly on chest radiography; and decreased LVEF on echocardiography. Cardiomegaly was defined as a cardiothoracic ratio of ≥ 0.55 (≥ 0.60 for patients aged less than 1 year) on chest radiography or LV end-diastolic diameter (LVDD) of $\geq 120\%$ of the normal value on echocardiography.

Patients were diagnosed with LVNC based on the following criteria defined by Ichida et al.: (1) two-layered myocardium with a noncompacted-to-compacted (N/C) ratio of more than 2.0 at end diastole, (2) prominent endomyocardial trabeculations that are distributed in more than one LV wall segment, and (3) deep fossas filled with blood from the ventricular cavity on color Doppler imaging [8]. All echocardiographic records were analyzed by two reviewers (K.H. and S.O.).

The thickness of the LV wall and N/C ratio (N; the depth of trabecular recesses. C; compacted wall thickness) were measured according to previously reported methods to quantify the extent of the trabecular meshwork [9,10]. The thickness of the compacted layer in the LV posterior wall (LVPWC) and LVDD are represented as z-scores based on the body surface area [11].

N/C ratios of 5 LV wall segments at end diastole; the anterior, lateral, and posterior walls; and interventricular septum at the level of the papillary muscles in the short-axis view and the apex in the long-axis view were measured [12–14].

2.5. Variant Screening

After obtaining informed consent from the patients or their parents, DNA was isolated from a whole-blood or heart tissue sample from each patient. Next-generation sequencing (NGS) was performed with 182 cardiac disorder-related genes (Table S1) using the Ion PGM System (Life Technologies, Carlsbad, CA, USA).

After all candidate pathogenic variants passed the selection criteria, to validate the result of NGS, Sanger sequencing was conducted.

2.6. Data Analysis and Variant Classification

The gnomAD database and Human Genetic Variation Database (HGVD), which contain data from 1208 Japanese individuals, was used to determine the allelic frequency of all detected variants. All variants were filtered with a minor allele frequency (MAF) of ≥ 0.0005 among the gnomAD and HGVD population. To evaluate the pathogenicity of the variants, seven different *in silico* predictive algorithms were used (Table S2). The pathogenicity of an identified variant was evaluated by the guidelines of the American College of Medical Genetics and Genomics [15].

2.7. Gene-Based Collapsing Test

A genic collapsing test was performed to confer risk genes of LVNC [16,17]. Fisher's exact test was conducted for each gene in collapsing analysis with a nominal significance level of $< 2.74 \times 10^{-4}$ for the number of assessable genes according to Bonferroni's correction.

2.8. Statistical Analysis

Continuous variables are presented as mean \pm standard deviation, and medians and ranges as appropriate. Categorical variables are given as frequencies and percentages. Statistical analyses were conducted with the use of the JMP software (version 13; SAS Institute, Cary, NC, USA). Receiver

operating characteristic curve analysis was performed to determine the optimum cutoff levels of the number of derivations obtained from electrocardiogram and echocardiographic data to predict MACEs. A *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Clinical and Cardiological Characteristics

Patient demographics and outcomes are shown in Table 1 and Figure 1. Overall, 53 patients (25 males and 28 females) were enrolled in this study. Their age at diagnosis ranged from 0 to 14 years (median: 0.3 months). The median follow-up period was 3.0 years (0.5–17.0 years). A total of 11 patients (20.8%) reported a family history of cardiomyopathy; 23 (43.4%) were diagnosed with CHF, 14 with heart murmur (26.4%), and 6 with cyanosis (11.3%). During the observation period, 31 patients (58.5%) experienced CHF and 13 (24.5%) developed arrhythmias such as ventricular tachycardia, supraventricular tachycardia, and atrioventricular block. No patient had a history of thrombosis. Other systemic malformations were observed in 10 patients (18.9%; Table S3).

Table 1. Comparison of physical findings of patients with LVNC and VSD.

| | LVNC with CHD (<i>n</i> = 53) | LVNC with VSD (<i>n</i> = 25) | VSD (<i>n</i> = 57) | <i>p</i> Value |
|-------------------------------|-----------------------------------|-----------------------------------|-------------------------|----------------|
| Demographic data | | | | |
| Male | 25 (47.1%) | 15 (60%) | 29 (50.9%) | 0.4724 |
| Age at diagnosis (m, median) | 0.27 (0–168) | 0.4 (0–60) | 0.10 (0–3) | 0.3659 |
| Duration of follow up (year) | 3.0 (0.5–17) | 3.0 (1.0–17) | 3.9 (0.5–12.2) | 0.6803 |
| Family history of CM | 11 (20.8%) | | | |
| Symptoms at diagnosis | | | | |
| Heart failure | 23 (43.4%) | 11 (44.0%) | 4 (7.0%) | 0.0002 |
| Heart murmur | 14 (26.4%) | 8 (32.0%) | 50 (87.7%) | <0.0001 |
| Cyanosis | 6 (11.3%) | 1 (4.0%) | 0 (0%) | 0.3086 |
| Fetal screening | 5 (9.4%) | 3 (12.0%) | 1 (1.8%) | 0.0822 |
| Neonatal screening | 4 (7.5%) | 1 (4.0%) | 2 (3.5%) | 0.6696 |
| Arrhythmia | 1 (1.9%) | 1 (4.0%) | 0 (0%) | 0.3049 |
| Current clinical presentation | | | | |
| Heart failure | 31 (58.5%) | | | |
| Embolism | 0 (0%) | | | |
| Arrhythmia | 13 (24.5%) | | | |
| VT | 5 (9.4%) | | | |
| SVT | 2 (3.8%) | | | |
| CAVB | 4 (7.5%) | | | |
| AFL | 2 (3.8%) | | | |
| Extracardiac abnormalities | 10 (18.9%) | | | |
| HF requiring hospitalization | 44 (83.0%) | | | |
| Age at surgery | 11.15 ± 14.78 | | | |
| Death | 4 (7.5%) | 1 (4.0%) | 0 (0%) | 0.2963 |
| Type of CHD | | | | |
| VSD | 29 (54.7%) | 25 (100%) | 57 (100%) | <0.0001 |
| ASD | 17 (32.1%) | | | |
| PDA | 10 (18.9%) | | | |
| Ebstein's disease | 7 (13.2%) | | | |
| DORV | 7 (13.2%) | | | |
| CoA | 4 (7.5%) | | | |
| PS | 4 (7.5%) | | | |
| AS | 2 (3.8%) | | | |
| hypo RV | 2 (3.8%) | | | |
| absent PV | 1 (1.9%) | | | |
| IAA | 1 (1.9%) | | | |

Table 1. Cont.

| | LVNC with CHD (n = 53) | LVNC with VSD (n = 25) | VSD (n = 57) | p Value |
|--------------------------|---------------------------|---------------------------|-----------------|---------|
| Heterotaxy syndrome | 1 (1.9%) | | | |
| MA | 1 (1.9%) | | | |
| PA | 1 (1.9%) | | | |
| TA | 1 (1.9%) | | | |
| TGA | 1 (1.9%) | | | |
| TOF | 1 (1.9%) | | | |
| TS | 1 (1.9%) | | | |
| Type of surgery | | | | |
| BVR | 19 (35.8%) | | | |
| PAB | 2 (3.8%) | | | |
| bil PAB | 2 (3.8%) | | | |
| PDA ligation | 2 (3.8%) | | | |
| PTPV | 2 (3.8%) | | | |
| PTAV | 1 (1.9%) | | | |
| PA debanding + PA plasty | 1 (1.9%) | | | |
| Electrocardiography | | | | |
| ST-segment depression | 3 (7.5%) | | | |
| T-wave abnormality | 8 (20.0%) | | | |
| Pathologic Q-wave | 7 (17.5%) | | | |
| LBBB | 3 (7.5%) | | | |
| RBBB | 10 (25.0%) | | | |
| fragmented QRS | 16 (40.0%) | | | |
| J wave | 7 (17.5%) | | | |
| LQT | 5 (12.5%) | | | |
| WPW syndrome | 1 (2.5%) | | | |

LVNC-CHD; left ventricular noncompaction with congenital heart disease, VSD; ventricular septal defect, CM; cardiomyopathy, VT; ventricular tachycardia, SVT; supra ventricular tachycardia, CAVB; complete AV block, AFL; atrial flutter, HF; heart failure, ASD; atrial septal defect, PDA; patent ductus arteriosus, Ebstein; Ebstein’s anomaly, DORV; double outlet of right ventricle, CoA; coarctation of aorta, PS; pulmonary valve stenosis, AS; aortic valve stenosis, hypo RV; hypoplastic right ventricle, PV; pulmonary valve, IAA; interruption of aortic arch, MA; mitral valve atresia, PA; pulmonary valve atresia, TA; tricuspid valve atresia, TGA; transposition of the great arteries, TOF; tetralogy of Fallot, TS; tricuspid valve stenosis, BVR; biventricular repair, PAB; pulmonary artery banding, bil PAB; bilateral pulmonary artery banding, PTPV; percutaneous transvenous pulmonary valvuloplasty, PTAV; percutaneous transvenous artery valvuloplasty, LBBB; left bundle branch block, RBBB; right bundle branch block, LQT; long QT. Continuous variables between the group of LVNC with VSD and the group of VSD were compared using the unpaired t-test, non-parametric Mann–Whitney test, or one-way analysis of variance, and categorical variables were compared using χ^2 statistics or Fisher’s exact test, as appropriate.

According to the type of CHD, 29 patients (54.7%) had VSDs, 17 (32.1%) had atrial septal defects, 10 had patent ductus arteriosus (PDA), and 7 (13.2%) had Ebstein’s anomaly and double outlet right ventricle.

A total of 30 patients underwent surgery at, on average, 11.2 months of age; 19 underwent biventricular repair (BVR); and 2 underwent pulmonary artery banding (PAB), bilateral PAB, and PDA ligation. Moreover, 13 patients (43.3%) were diagnosed with LVNC postoperatively (Figure 1).

Upon electrography, fragmented QRS was frequently observed in 16 patients (40.0%), followed by right bundle branch block (25.0%), T-wave abnormality (20.0%), Q wave (17.5%), J wave (17.5%), ST-segment depression (12.3%), and long QT syndrome (12.5%).

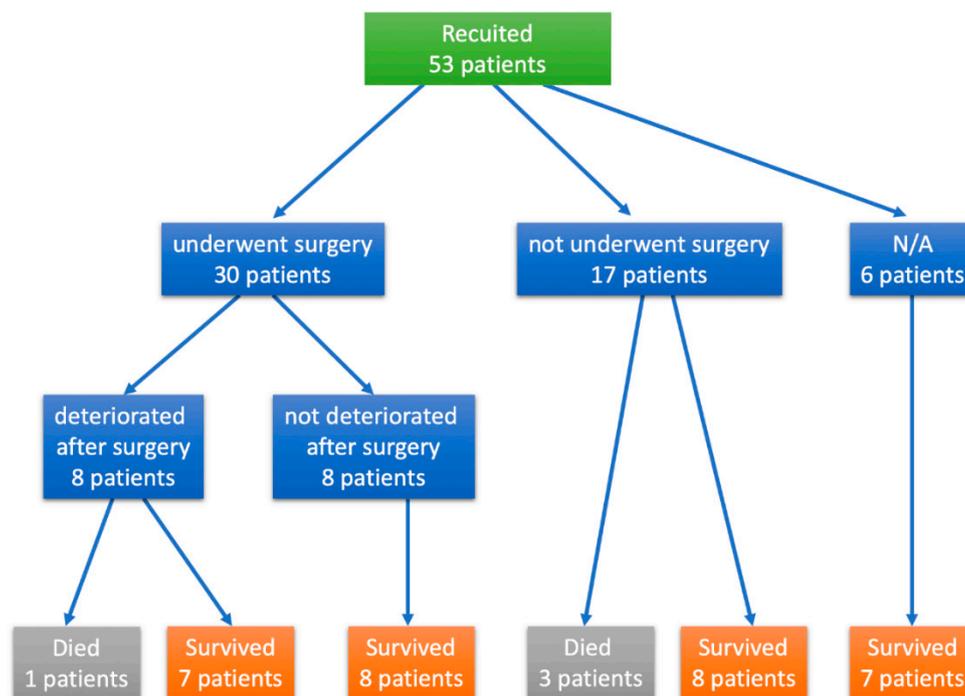


Figure 1. Flowchart of included and excluded patients. Thirty patients underwent surgery and the condition of 8 worsened postoperatively. Adverse events were noted in 4 patients.

3.2. Genetic Analysis

After excluding common polymorphisms on the basis of variant frequencies reported in gnomAD and HGVD and *in silico* analysis predictions, we identified 30 rare exonic (25 missense and 2 frameshift indel) and 3 splice site variants classified as pathogenic or likely pathogenic (Table 2) in 28 patients with LVNC and CHD. Eight variants were detected in *MYH7*, two in *TPM1*, and one in *ACTC1*, *ANK2*, *COL4A1*, *DAAM1*, *DSG2*, *DSP*, *FGF16*, *FGFR2*, *HCN4*, *JUP*, *MYBPC3*, *MYH6*, *MYL2*, *PKP2*, *PRDM16*, *RYR2*, and *TAZ* each. Sarcomere gene variants accounted for 50.0%. All variants affected conserved amino acid residues. In addition, the genetic collapsing test showed that variants in *MYH7* ($p = 2.104 \times 10^{-16}$, ranked first) and *TPM1* ($p = 1.356 \times 10^{-4}$, ranked second) reached significance (adjusted alpha = $p < 2.74 \times 10^{-4}$), which strongly suggested that variants in both genes increase the risk of LVNC (Table S4).

Table 2. Variants identified in patients with LVNC.

| Patient Number | Gene | Protein | Coding | dbSNP | gnomAD | gnomAD (EA) | HGVD | Fathmm | Sift | Polyphen2 | GVGD | Mutation Taster | Provean | CADD | Clin Var |
|----------------|--------|--------------|------------------|-------------|-------------|-------------|------|--------|-------|-----------|------|-----------------|---------|--------|------------------------|
| 1 | LDB3 | p.Thr282Met | c.845C > T | n/a | 0.00001991 | 0.0001088 | n/a | n/a | 0 | 1 | C0 | 1 | -0.28 | -4.91 | uncertain significance |
| 2 | DSP | p.Arg907Cys | c.2719C > T | n/a | 0.00003185 | 0 | n/a | -3.43 | 0 | 0.999 | C0 | 1 | 1.96 | -3.58 | uncertain significance |
| 2 | PKP2 | p.Thr50fs | c.148_151delACAG | rs397517067 | n/a | n/a | n/a | -4.54 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| 6 | JUP | p.Arg233Pro | c.698G > C | n/a | n/a | n/a | n/a | -4.39 | 0.03 | 0.999 | C0 | 1 | 1.03 | -4.36 | n/a |
| 9 | MYL2 | p.Glu88Lys | c.262G > A | rs753032598 | n/a | n/a | n/a | -0.23 | 0.017 | 0.996 | C15 | 1 | -1.15 | -3.62 | n/a |
| 11 | MYH7 | p.Arg712His | c.2135G > A | rs199473346 | n/a | n/a | n/a | -5.47 | 0 | 0.988 | C25 | 1 | -4.54 | -4.35 | n/a |
| 16 | MYH7 | p.Met362Arg | c.1085T > G | rs199473346 | n/a | n/a | n/a | -5.47 | 0 | 0.001 | 0 | 1 | -3.64 | -5.15 | n/a |
| 18 | MYH7 | p.Met362Arg | c.1085T > G | rs199473346 | n/a | n/a | n/a | -5.47 | 0 | 0.001 | 0 | 1 | -3.64 | -5.15 | n/a |
| 20 | MYBPC3 | p.Arg891Trp | c.2671C > T | rs200229074 | 0.0000271 | 0.0001996 | n/a | -1.83 | 0.001 | 1 | C65 | 1 | 0.37 | -6.52 | uncertain significance |
| 21 | TPM1 | p.Arg238Gln | c.713G > A | n/a | n/a | n/a | n/a | -3.32 | 0.001 | 0.999 | C35 | 1 | -6.36 | -3.22 | n/a |
| 23 | MYH7 | - | c.896-1C > T | n/a | n/a | n/a | n/a | -4.49 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| 24 | MYH7 | p.Phe230Ser | c.689T > C | n/a | n/a | n/a | n/a | 5.9 | 0 | 0.984 | 0 | 1 | -4.96 | -6.07 | n/a |
| 25 | DAAM1 | p.Ala187fs | c.557_558insA | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | - | - | n/a |
| 27 | MYH7 | p.Leu693Arg | c.2078T > G | rs749051278 | n/a | n/a | n/a | 1.96 | 0 | 0.997 | C65 | 1 | -4.85 | -5.29 | n/a |
| 28 | MYH7 | p.Leu620Pro | c.1859T > C | n/a | 0.000003977 | 0 | n/a | n/a | 7 | 0.969 | C65 | 1 | -4.06 | -6.25 | n/a |
| 30 | MYH7 | p.Arg23Trp | c.67C > T | rs749297714 | 0.00002475 | 0 | n/a | 3.31 | 0.002 | 0.997 | 0 | 1 | -2.08 | -4.22 | uncertain significance |
| 31 | PRDM16 | p.Ser723fs | c.2168delC | n/a | 0.00001071 | 0 | n/a | -2.31 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| 33 | TPM1 | p.Arg238Gln | c.713G > A | n/a | n/a | n/a | n/a | n/a | 0.001 | 0.999 | C35 | 1 | -6.36 | -3.22 | n/a |
| 34 | FGF16 | - | c.105 + 4AG > GT | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| 34 | FGFR2 | - | c.939 + 40T > C | n/a | 0.0000533 | 0.0004516 | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| 35 | COL4A1 | p.Pro108Ser | c.322C > T | rs769020772 | n/a | n/a | n/a | n/a | 0 | 0.999 | C65 | 1 | 5.9 | -5.28 | n/a |
| 36 | RYR2 | p.Leu4597Ser | c.13790T > C | n/a | n/a | n/a | n/a | n/a | 0 | 0.999 | C0 | 1 | -5.09 | -4.614 | n/a |
| 37 | ACTC1 | p.Arg212His | c.635G > A | rs121908411 | n/a | n/a | n/a | -7.52 | 0 | 0.887 | C25 | 1 | -4.54 | -3.896 | uncertain significance |
| 38 | TNNT2 | p.Lys298Thr | c.893A > C | rs121908411 | n/a | n/a | n/a | -7.52 | 0 | 0.999 | C0 | 1 | 2.19 | -3.071 | n/a |
| 43 | ANK2 | p.Arg895Gln | c.2684G > A | rs146581757 | 0.00004773 | 0.0002719 | 0.04 | -0.23 | 0.05 | 0.998 | C0 | 1 | -0.3 | -3.277 | n/a |
| 45 | TAZ | p.Gly216Arg | c.646G > A | n/a | n/a | n/a | n/a | 1.03 | 0.05 | 1 | C0 | 1 | -3.5 | -6.733 | n/a |
| 46 | MYH6 | p.Glu1713Lys | c.5137G > A | rs121908441 | 0.00003197 | 0.00005013 | n/a | -2.4 | 0 | 1 | C55 | 1 | -1.84 | -2.522 | uncertain significance |
| 47 | DSG2 | p.Tyr235His | c.703T > C | rs199472921 | n/a | n/a | n/a | -4.93 | 0 | 1 | C65 | 1 | -0.41 | -4.678 | n/a |
| 48 | COL4A1 | p.Gln462Arg | c.1385A > G | rs147445322 | 0.00001991 | 0.0002718 | 0.04 | -6.37 | 0.04 | 0.491 | C0 | 1 | -3.19 | -1.633 | n/a |
| 50 | HCN4 | p.Asp432His | c.1294G > C | rs147445322 | n/a | n/a | n/a | -2.61 | 0.12 | 1 | C0 | 1 | -4.92 | -5.923 | n/a |

3.3. Cardiological Characteristics

Echocardiography showed lower EFs and more thickened trabeculations in LV in children with LVNC and CHD than in those with VSDs (Table 3 and Figure 2). The average LV posterior wall (LVPW) z-score at the last visit was significantly higher than that at the initial visit ($p = 0.0482$). The average z-score of LVPWC thickness at the last visit was significantly lower than that of the initial visit ($p = 0.0061$).

Table 3. Comparison of physical findings of patients with LVNC and VSD between the initial and last visit.

| | LVNC with VSD (n = 21) | | | VSD (n = 57) | | |
|-------------------------------|---------------------------|---------------|---------|-----------------|---------------|---------|
| | Initial | Last | p Value | Initial | Last | p Value |
| Age | 5.43 ± 13.24 | 56.25 ± 76.97 | 0.2768 | 19.05 ± 28.26 | 33.39 ± 28.32 | <0.0001 |
| Cardiac function at diagnosis | | | | | | |
| LVEF (%) | 59.00 ± 12.97 | 57.85 ± 14.73 | 0.7192 | 70.09 ± 8.29 | 68.47 ± 5.35 | 0.1363 |
| LVDD Z-score | 1.323 ± 3.16 | 1.14 ± 2.31 | 0.9576 | 2.02 ± 1.74 | 0.19 ± 1.00 | <0.0001 |
| LVPW Z-score | 6.13 ± 1.57 | 4.72 ± 1.84 | 0.0482 | 2.73 ± 1.78 | 2.13 ± 1.53 | 0.0814 |
| LVPWC Z-score | -3.13 ± 2.22 | -5.48 ± 2.11 | 0.0061 | -2.67 ± 2.07 | -2.78 ± 1.89 | 0.5709 |
| N/C ratio | | | | | | |
| Anterior wall | -3.13 ± 2.22 | -5.48 ± 2.11 | 0.5370 | 0.50 ± 0.31 | 0.38 ± 0.18 | 0.0632 |
| Septal wall | -3.13 ± 2.22 | -5.48 ± 2.11 | 0.1723 | 0.41 ± 0.20 | 0.29 ± 0.11 | 0.0015 |
| Lateral wall | -3.13 ± 2.22 | -5.48 ± 2.11 | 0.9385 | 0.73 ± 0.44 | 0.72 ± 0.37 | 0.8735 |
| Posterior wall | 3.21 ± 1.45 | 3.81 ± 1.50 | 0.2028 | 1.30 ± 0.50 | 1.14 ± 0.49 | 0.0846 |
| Apex | 3.45 ± 1.72 | 3.97 ± 1.62 | 0.1913 | 1.52 ± 0.88 | 4.22 ± 1.62 | <0.0001 |
| Mean 5 segments | 2.13 ± 0.84 | 2.19 ± 0.61 | 0.4897 | 0.89 ± 0.24 | 1.35 ± 0.38 | <0.0001 |

LVNC-CHD; left ventricular noncompaction with congenital heart disease, VSD; ventricular septal defect, LVEF; left ventricular ejection fraction, LVDD; left ventricular diastolic dimension, LVPW; left ventricular posterior wall, LVPWC; compacted layer of left ventricular posterior wall, N/C; ratio of noncompacted/compacted layer.

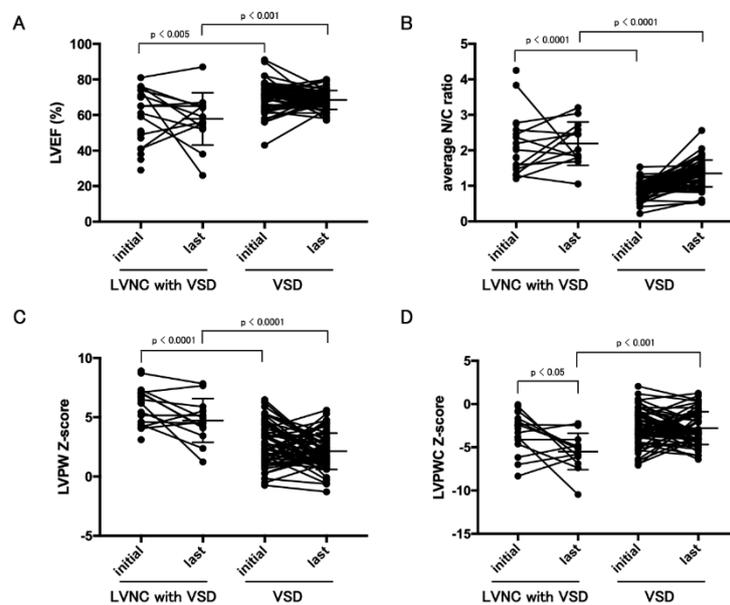


Figure 2. Longitudinal data of echocardiographic data between LVNC with CHD and VSD groups. LVEF (A), average N/C ratio (B), LVPW z-score (C), and LVPWC z-score (D) between the initial and last visits. LVNC; left ventricular noncompaction, VSD; ventricular septal defect, LVEF; left ventricular ejection fraction, N/C; ratio of noncompacted/compacted layer, LVPW; left ventricular posterior wall, LVPWC; compacted layer of left ventricular posterior wall.

3.4. Characteristics of Patients with Adverse Events

Adverse events were observed in 4 patients, and 4 patients died (Table 4 and Figure 1). Although cardiac death, LV assist device implantation, HT, and ICD shock were classified as MACEs in this study, none of the patients underwent HT or LV assist device implantation or experienced ICD shock. Aside from 1 patient, 3 died at early infancy and never underwent surgery.

The condition of 8 patients worsened postoperatively (Table 5 and Figure 1), all patients had VSDs, 3 had variants in *MYH7*, 6 underwent BVR, and long-term medical therapy were required in all patients for myocardial dysfunction after their latest surgeries.

The analysis of the multivariable proportional hazards model revealed that CHF during follow-up, LVEF of < 24%, LVDD z-score of > 8.56, and N/C ratio of the LV apex of > 8.33 at the last visit were risk factors for survival without MACE occurrence (Table 6). Patients with LVNC and CHD had a worse prognosis than those with VSDs (Figure 3).

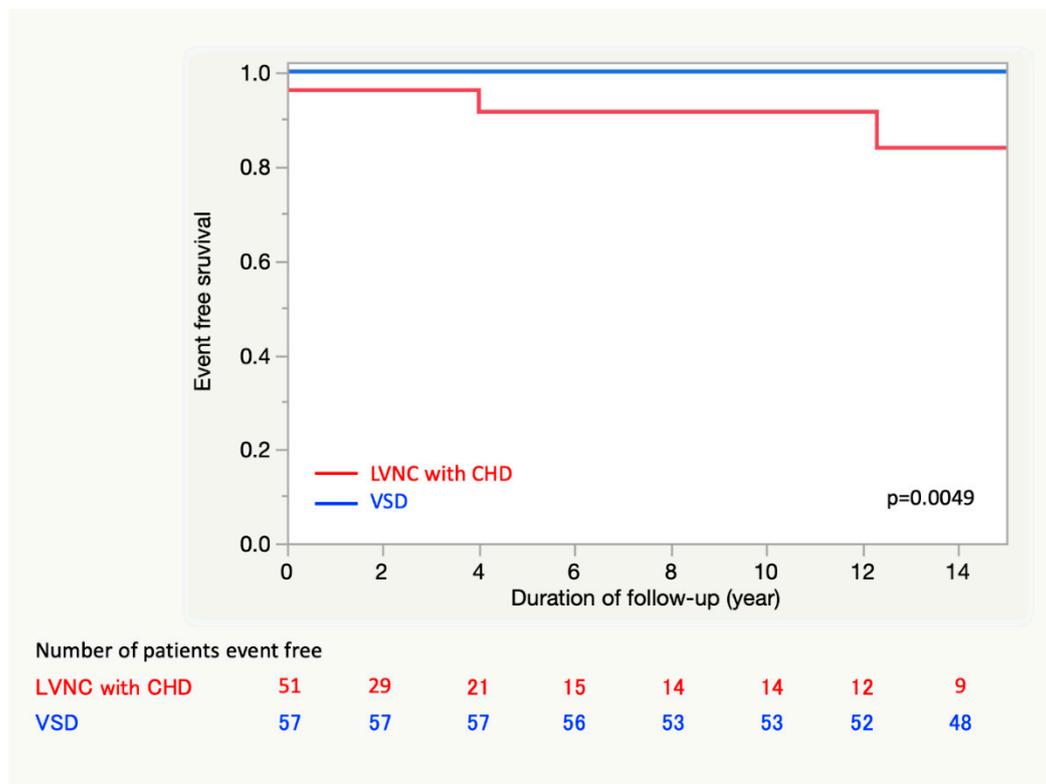


Figure 3. Event-free survival to the endpoint of major adverse cardiac events in LVNC with CHD and VSD groups. LVNC; left ventricular noncompaction, VSD; ventricular septal defect.

Table 4. Summary of death cases.

| ID | Sex | Age at Diagnosis | Symptoms at Diagnosis | CHD | Variants | FH of CM | Extracardiac Abnormalities | Current Clinical Presentation | Age at Death | Cause of Death |
|----|-----|------------------|-----------------------|----------------|-------------|----------|----------------------------|-------------------------------|--------------|----------------|
| 13 | F | 1 d | bradycardia | VSD | - | yes | no | CHF | 1d | shock |
| 43 | M | 0 d | CHF | DORV, TGA, VSD | <i>ANK2</i> | no | no | CHF | 12y | CHF |
| 45 | M | 0 d | CHF | ASD, PDA | <i>TAZ</i> | no | no | CHF | 2m | shock |
| 52 | M | fetus | CHF | Ebstein | - | no | no | CHF | 0d | shock |

CHD; congenital heart disease, FH; family history, CM; cardiomyopathy, VSD; ventricular septal defect, DORV; double outlet of right ventricle, Ebstein; Ebstein’s anomaly, ASD; atrial septal disease, TVD; tricuspid valve dysplasia. BVR; biventricular repair, CHF; congestive heart failure.

Table 5. Summary of deteriorated cases postoperatively.

| ID | Sex | Age at Diagnosis | Symptoms at Diagnosis | CHD | Variants | FH of CM | Extracardiac Abnormalities | Type of Surgery | Age at Surgery | Current Clinical Presentation | Outcome |
|----|-----|------------------|-----------------------|-------------------|-------------|----------|----------------------------|--------------------------|----------------|-------------------------------|---------|
| 5 | M | 1 d | cyanosis | DORV, VSD, IAA | - | no | no | BVR | 5 | CHF | alive |
| 16 | F | fetus | cyanosis | Ebstein, VSD, CoA | <i>MYH7</i> | yes | no | BVR | 1 | CHF | alive |
| 17 | F | 1 y | CHF | VSD | - | no | no | BVR | 16 | CHF | alive |
| 18 | F | 4 d | heart murmur | Ebstein, VSD, CoA | <i>MYH7</i> | yes | no | BVR | 0 | CHF | alive |
| 23 | F | fetus | CHF | DORV, VSD, IAA | <i>MYH7</i> | yes | no | bilPAB | 0 | CHF | alive |
| 43 | M | 0 d | cyanosis | TGA, DORV, VSD | <i>ANK2</i> | no | no | BVR | 17 | CHF | death |
| 47 | F | 12 d | heart murmur | ASD, VSD, PDA | <i>DSG2</i> | no | chromosome 12 abnormality | PA debanding + PA plasty | 34 | CHF | alive |
| 53 | M | 1 m | CHF | VSD | - | no | no | BVR | 1 | no | alive |

CHD; congenital heart disease, FH; family history, CM; cardiomyopathy, DORV; double outlet of right ventricle, VSD; ventricular septal defect, IAA; interruption of aortic arch, Ebstein; Ebstein’s anomaly, CoA; coarctation of aorta, TGA; transposition of the great arteries, ASD; atrial septal disease, PDA; patent ductus arteriosus, BVR; biventricular repair, PAB; pulmonary artery banding, bil PAB; bilateral pulmonary artery banding.

Table 6. Univariate analysis of risk factors for death in the patients with LVNC.

| Variable | Univariable Survival Analysis | |
|-------------------------------|--|---------|
| | Odds Ratio (95% CI) | p Value |
| Male | 3.54 (0.42–74.51) | 0.2536 |
| Family history | 4.33 (0.47–40.33) | 0.1809 |
| Heart failure at diagnosis | 4.39×10^7 (0.62–) | 0.1049 |
| Heart failure | 8.06×10^6 (1.13–) | 0.0404 |
| Extracardiac abnormalities | 4.75 (0.51–44.78) | 0.1586 |
| Gene variants | 0.85 (0.96–7.52) | 0.8726 |
| Double variants | 2.87×10^{-6} (-5.89×10^{-121}) | 0.5676 |
| UCG parameters at first visit | | |
| LVEF < 50% | 0.89 (0.098–8.13) | 0.9160 |
| UCG parameters at last visit | | |
| LVEF < 24% | 1.84×10^{15} (0.0051–10.55) | 0.0051 |
| LVDD Z score > 8.56 | 1.84×10^{15} (0.0054–9.95) | 0.0054 |
| N/C ratio of apex > 8.33 | 1.84×10^{15} (0.0051–10.55) | 0.0051 |

CI, confidence interval, UCG; cardiac ultrasound, LVEF; left ventricular ejection fraction, LVDD; left ventricular diastolic dimension, N/C; ratio of noncompacted/compacted layer.

4. Discussion

LVNC is associated with CHD, ranging from PDA or atrial septal defects/VSDs to more severe diseases such as Ebstein's anomaly [4]. Our study demonstrated three features: (1) pathogenic variants were identified in more than half of the patients; (2) patients with LVNC had lower EFs than those with VSDs throughout the study period; and (3) postoperative deterioration was observed in several patients.

A variety of genetic disorders are associated with LVNC, including Z-disk and sarcomere gene variants, mitochondrial disorders, and ion channel gene variants [18–22]. Thus, structural congenital malformations and impaired LV myocardial differentiation may be caused by genetic abnormalities. Additionally, for the development of LVNC in a patient with genetic variants, remarkable change of hemodynamic circulation in the fetus may be a cofactor [23]. In our results, variants in *MYH7* were most commonly identified and the variants significantly increase the risk of LVNC by rare variant collapsing analysis. The mechanisms by which variants in the *MYH7* gene induce LVNC remain unclear. Analyzing the positions of these variants against their amino acid location showed several hotspots wherein variants are more popular, which seemed to tend to be in key functional locations. We observed that all variants in *MYH7* associated with LVNC were found in the segment 1 domain. Moreover, enrichment of pathogenic variants was observed in the crucial functional domains of the ATP-binding domain [24–26]. It suggested that the majority of the identified variants affect the force output by affecting either regulation of the ATPase cycle, movement of the lever, or interaction between myosin and actin. Remarkably, the location of variants in *MYH7* in LVNC was different from that in hypertrophic cardiomyopathy (HCM) patients. Hotspots of HCM were mostly located in the surface spanning the converter domain and the myosin mesa; the flat surface of the myosin catalytic domain [27]. It is important to distinguish variant types and assess them in light of well-known disease mechanisms. Therefore, to understand the pathophysiology and development of LVNC, it is critically notable for the patients with LVNC and CHD to characterize genetic variants and phenotypic abnormalities.

Children with LVNC and CHD have a higher incidence of CHF than patients with VSDs. Additionally, our results showed that the condition of 8 of the 30 patients (26.7%) worsened postoperatively, whether palliative or radical. The underlying pathophysiologic mechanisms of CHF remain unclarified. Systolic dysfunction in LVNC is believed to be due to subendocardial hypoperfusion [1,28]. It is also believed that diastolic dysfunction occurs by a restrictive filling pattern and abnormal relaxation because of the presence of LV hypertrabeculation [29]. These speculations are based on the evidence that the noncompact layer has typically low perfusion, which has been

demonstrated on multiple modalities [30]. Functionally, LV torsion is more common in patients with LVNC [31]. LV twist is generated by the movement of two orthogonally oriented muscular bands of a helical myocardial structure concomitant with a clockwise rotation of the base and counterclockwise rotation of the apex in LV [32]. Van Dalen et al. used two-dimensional speckle tracking echocardiography and demonstrated that LV basal and apical rotation are in the same direction, resulting in a lack of LV twist in patients with LVNC [33]. Bellavia et al. reported that in adults, the value of LV rotation/torsion excessively decreased in patients with LVNC, whereas normal EF were retained when compared with those in controls [31]. Nawaytow et al. reported that almost half of the children with LVNC exhibit reverse apical rotation, resulting in decreased LV torsion and untwist rate, which are associated with the degree of LVNC [34]. These previous studies might support that the deterioration of LV function occurs during the perioperative period, although LV systolic function was preserved preoperatively because of its unique structure.

In our study, 13 patients were diagnosed with LVNC postoperatively. The existence of additional triggers such as dynamic hemodynamic changes during the perioperative period were suggested. Indeed, the etiology of LVNC remains unknown. One possibility is that primary abnormality in early myocardial morphogenesis may cause LVNC. Another possibility is that prenatal or postnatal additional triggers such as pressure overload on the LV may cause LVNC. LVNC in the setting of CHD may be one of the models where both hemodynamic and genetic factors interact with each other, resulting in abnormal LV differentiation. Thus, additional stress to the myocardium may trigger the worsening of systolic function in patients with LVNC and CHD because LVNC is more frequently associated with systolic dysfunction than that of CHD without LVNC.

There were no predictors of postoperative CHF in this study. Preoperative preserved systolic function did not predict the outcome of patients with LVNC and CHD. In fact, not all patients with LVNC had systolic dysfunction during the preoperative evaluation. Most patients with LVNC and CHD had mildly depressed systolic function preoperatively. These facts may complicate the establishment of medical treatment during the operative period and optimal timing of surgery.

In our study, patients with LVNC and CHD were observed to have a higher frequency of arrhythmias (24.5%). Recently, it was demonstrated in pediatric patients that LVNC with associated CHD confers additional risk [35]. Although there were no data relationships between mortality and the prevalence of arrhythmias in patients with LVNC and CHD, our data suggest that more attention should be paid to the occurrence of arrhythmia and CHF because of the higher prevalence of these symptoms.

Limitations

The number of patients in our study was small. We were not able to track patients for a long period of time, particularly those who were referred from external facilities, because this was a retrospective study. This study included data from over approximately 15 years. During this period, the development of disease-modifying treatments was improved, which may have altered study outcomes. The small number of patients with genetic variants also has a limitation regarding the significance of the association with variants and prognosis. To clarify the effect of variants on clinical manifestations and prognosis, further analyses of larger numbers of patients are required. Additionally, functional analyses are also required to clarify the significance of the identified variants which contribute to the etiology of LVNC.

5. Conclusions

To the best of our knowledge, this is the first large cohort study to reveal the etiology and genetic background of LVNC and CHD. Recognition of the association between LVNC and CHD is crucial considering the increased risk of CHF as demonstrated in our results. Moreover, our data suggest that concomitant LVNC with CHD is a surgical risk factor, so additional perioperative planning such as CHF treatment may be beneficial if identified preoperatively. Therefore, elucidation of genotype-phenotype

correlation in patients with LVNC and CHD may be important to understand the pathophysiology and development of LVNC in patients with CHD. Further studies will continue to determine long-term and genotype–phenotype correlations.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2077-0383/9/3/785/s1>, Table S1: List of 182 analyzed genes of NGS, Table S2: Silico predictive algorithms used in the study, Table S3: List of patients with extracardiac anomaly, and Table S4: Gene collapsing test of rare variants.

Author Contributions: Conceptualization, K.H.; methodology, Y.H.; validation, N.N. and N.Y.; formal analysis, Y.H.; investigation, S.T. and H.N. and K.I. and S.O.; data curation, M.O.; writing—original draft preparation, K.H.; writing—review and editing, K.H.; visualization, N.M.; supervision, F.I.; project administration, K.H.; All authors have read and agreed to the published version of the manuscript.

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Abbreviations

| | |
|------|--------------------------------|
| LVNC | left ventricular noncompaction |
| LV | left ventricle |
| CHD | congenital heart disease |
| CHF | congestive heart failure |

References

1. Chin, T.K.; Perloff, J.K.; Williams, R.G.; Jue, K.; Mohrmann, R. Isolated noncompaction of left ventricular myocardium. A study of eight cases. *Circulation* **1990**, *82*, 507–513. [[CrossRef](#)] [[PubMed](#)]
2. Jefferies, J.L.; Wilkinson, J.D.; Sleeper, L.A.; Colan, S.D.; Lu, M.; Pahl, E.; Kantor, P.F.; Everitt, M.D.; Webber, S.A.; Kaufman, B.D.; et al. Cardiomyopathy Phenotypes and Outcomes for Children With Left Ventricular Myocardial Noncompaction: Results From the Pediatric Cardiomyopathy Registry. *J. Card. Fail.* **2015**, *21*, 877–884. [[CrossRef](#)] [[PubMed](#)]
3. Towbin, J.A.; Jefferies, J.L. Cardiomyopathies Due to Left Ventricular Noncompaction, Mitochondrial and Storage Diseases, and Inborn Errors of Metabolism. *Circ. Res.* **2017**, *121*, 838–854. [[CrossRef](#)]
4. Stahli, B.E.; Gebhard, C.; Biaggi, P.; Klaassen, S.; Valsangiacomo Buechel, E.; Attenhofer Jost, C.H.; Jenni, R.; Tanner, F.C.; Greutmann, M. Left ventricular non-compaction: Prevalence in congenital heart disease. *Int. J. Cardiol.* **2013**, *167*, 2477–2481. [[CrossRef](#)] [[PubMed](#)]
5. Attenhofer Jost, C.H.; Connolly, H.M. Left ventricular non-compaction: Dreaming of the perfect diagnostic tool. *Eur. J. Heart Fail.* **2012**, *14*, 113–114. [[CrossRef](#)] [[PubMed](#)]
6. Antzelevitch, C.; Yan, G.X. J wave syndromes. *Heart Rhythm* **2010**, *7*, 549–558. [[CrossRef](#)] [[PubMed](#)]
7. Das, M.K.; Saha, C.; El Masry, H.; Peng, J.; Dandamudi, G.; Mahenthiran, J.; McHenry, P.; Zipes, D.P. Fragmented QRS on a 12-lead ECG: A predictor of mortality and cardiac events in patients with coronary artery disease. *Heart Rhythm* **2007**, *4*, 1385–1392. [[CrossRef](#)]
8. Ichida, F. Left ventricular noncompaction—Risk stratification and genetic consideration. *J. Cardiol.* **2019**. [[CrossRef](#)]
9. Jenni, R.; Rojas, J.; Oechslin, E. Isolated noncompaction of the myocardium. *N. Engl. J. Med.* **1999**, *340*, 966–967. [[CrossRef](#)]
10. Wald, R.; Veldtman, G.; Golding, F.; Kirsh, J.; McCrindle, B.; Benson, L. Determinants of outcome in isolated ventricular noncompaction in childhood. *Am. J. Cardiol.* **2004**, *94*, 1581–1584. [[CrossRef](#)]
11. Kampmann, C.; Wiethoff, C.M.; Wenzel, A.; Stolz, G.; Betancor, M.; Wippermann, C.F.; Huth, R.G.; Habermehl, P.; Knuf, M.; Emschermann, T.; et al. Normal values of M mode echocardiographic measurements of more than 2000 healthy infants and children in central Europe. *Heart* **2000**, *83*, 667–672. [[CrossRef](#)] [[PubMed](#)]

12. Pignatelli, R.H.; McMahon, C.J.; Dreyer, W.J.; Denfield, S.W.; Price, J.; Belmont, J.W.; Craigen, W.J.; Wu, J.; El Said, H.; Bezold, L.L.; et al. Clinical characterization of left ventricular noncompaction in children: A relatively common form of cardiomyopathy. *Circulation* **2003**, *108*, 2672–2678. [[CrossRef](#)] [[PubMed](#)]
13. Oechslin, E.N.; Attenhofer Jost, C.H.; Rojas, J.R.; Kaufmann, P.A.; Jenni, R. Long-term follow-up of 34 adults with isolated left ventricular noncompaction: A distinct cardiomyopathy with poor prognosis. *J. Am. Coll. Cardiol.* **2000**, *36*, 493–500. [[CrossRef](#)]
14. Wang, C.; Takasaki, A.; Watanabe Ozawa, S.; Nakaoka, H.; Okabe, M.; Miyao, N.; Saito, K.; Ibuki, K.; Hirono, K.; Yoshimura, N.; et al. Long-Term Prognosis of Patients With Left Ventricular Noncompaction-Comparison Between Infantile and Juvenile Types. *Circ. J.* **2017**, *81*, 694–700. [[CrossRef](#)] [[PubMed](#)]
15. Richards, S.; Aziz, N.; Bale, S.; Bick, D.; Das, S.; Gastier-Foster, J.; Grody, W.W.; Hegde, M.; Lyon, E.; Spector, E.; et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **2015**, *17*, 405–424. [[CrossRef](#)]
16. Cirulli, E.T.; Lasseigne, B.N.; Petrovski, S.; Sapp, P.C.; Dion, P.A.; Leblond, C.S.; Couthouis, J.; Lu, Y.F.; Wang, Q.; Krueger, B.J.; et al. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* **2015**, *347*, 1436–1441. [[CrossRef](#)]
17. Bagnall, R.D.; Crompton, D.E.; Petrovski, S.; Lam, L.; Cutmore, C.; Garry, S.I.; Sadleir, L.G.; Dibbens, L.M.; Cairns, A.; Kivity, S.; et al. Exome-based analysis of cardiac arrhythmia, respiratory control, and epilepsy genes in sudden unexpected death in epilepsy. *Ann. Neurol.* **2016**, *79*, 522–534. [[CrossRef](#)]
18. Stollberger, C.; Wegner, C.; Benatar, A.; Chin, T.K.; Dangel, J.; Majoor-Krakauer, D.; Mondal, T.K.; Sivanandam, S.; Silverman, N.H.; van Waning, J.; et al. Postnatal Outcome of Fetal Left Ventricular Hypertrabeculation/Noncompaction. *Pediatr. Cardiol.* **2016**, *37*, 919–924. [[CrossRef](#)]
19. Arbustini, E.; Weidemann, F.; Hall, J.L. Left ventricular noncompaction: A distinct cardiomyopathy or a trait shared by different cardiac diseases? *J. Am. Coll. Cardiol.* **2014**, *64*, 1840–1850. [[CrossRef](#)]
20. Hoedemaekers, Y.M.; Caliskan, K.; Michels, M.; Frohn-Mulder, I.; van der Smagt, J.J.; Phefferkorn, J.E.; Wessels, M.W.; ten Cate, F.J.; Sijbrands, E.J.; Dooijes, D.; et al. The importance of genetic counseling, DNA diagnostics, and cardiologic family screening in left ventricular noncompaction cardiomyopathy. *Circ. Cardiovasc. Genet.* **2010**, *3*, 232–239. [[CrossRef](#)]
21. Dellefave, L.M.; Pytel, P.; Mewborn, S.; Mora, B.; Guris, D.L.; Fedson, S.; Waggoner, D.; Moskowitz, I.; McNally, E.M. Sarcomere mutations in cardiomyopathy with left ventricular hypertrabeculation. *Circ. Cardiovasc. Genet.* **2009**, *2*, 442–449. [[CrossRef](#)]
22. Wang, C.; Hata, Y.; Hirono, K.; Takasaki, A.; Ozawa, S.W.; Nakaoka, H.; Saito, K.; Miyao, N.; Okabe, M.; Ibuki, K.; et al. A Wide and Specific Spectrum of Genetic Variants and Genotype-Phenotype Correlations Revealed by Next-Generation Sequencing in Patients with Left Ventricular Noncompaction. *J. Am. Heart Assoc.* **2017**, *6*. [[CrossRef](#)]
23. Sedmera, D.; Pexieder, T.; Vuillemin, M.; Thompson, R.P.; Anderson, R.H. Developmental patterning of the myocardium. *Anat. Rec.* **2000**, *258*, 319–337. [[CrossRef](#)]
24. Moore, J.R.; Leinwand, L.; Warshaw, D.M. Understanding cardiomyopathy phenotypes based on the functional impact of mutations in the myosin motor. *Circ. Res.* **2012**, *111*, 375–385. [[CrossRef](#)] [[PubMed](#)]
25. Buvoli, M.; Hamady, M.; Leinwand, L.A.; Knight, R. Bioinformatics assessment of beta-myosin mutations reveals myosin's high sensitivity to mutations. *Trends Cardiovasc. Med.* **2008**, *18*, 141–149. [[CrossRef](#)] [[PubMed](#)]
26. Rayment, I.; Holden, H.M.; Sellers, J.R.; Fananapazir, L.; Epstein, N.D. Structural interpretation of the mutations in the beta-cardiac myosin that have been implicated in familial hypertrophic cardiomyopathy. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 3864–3868. [[CrossRef](#)] [[PubMed](#)]
27. Spudich, J.A. The myosin mesa and a possible unifying hypothesis for the molecular basis of human hypertrophic cardiomyopathy. *Biochem. Soc. Trans.* **2015**, *43*, 64–72. [[CrossRef](#)] [[PubMed](#)]
28. Weiford, B.C.; Subbarao, V.D.; Mulhern, K.M. Noncompaction of the ventricular myocardium. *Circulation* **2004**, *109*, 2965–2971. [[CrossRef](#)] [[PubMed](#)]
29. Agmon, Y.; Connolly, H.M.; Olson, L.J.; Khandheria, B.K.; Seward, J.B. Noncompaction of the ventricular myocardium. *J. Am. Soc. Echocardiogr.* **1999**, *12*, 859–863. [[CrossRef](#)]
30. Soler, R.; Rodriguez, E.; Monserrat, L.; Alvarez, N. MRI of subendocardial perfusion deficits in isolated left ventricular noncompaction. *J. Comput. Assist. Tomogr.* **2002**, *26*, 373–375. [[CrossRef](#)]

31. Bellavia, D.; Michelena, H.I.; Martinez, M.; Pellikka, P.A.; Bruce, C.J.; Connolly, H.M.; Villarraga, H.R.; Veress, G.; Oh, J.K.; Miller, F.A. Speckle myocardial imaging modalities for early detection of myocardial impairment in isolated left ventricular non-compaction. *Heart* **2010**, *96*, 440–447. [[CrossRef](#)] [[PubMed](#)]
32. Kocica, M.J.; Corno, A.F.; Carreras-Costa, F.; Ballester-Rodes, M.; Moghbel, M.C.; Cueva, C.N.; Lackovic, V.; Kanjuh, V.I.; Torrent-Guasp, F. The helical ventricular myocardial band: Global, three-dimensional, functional architecture of the ventricular myocardium. *Eur. J. Cardiothorac. Surg.* **2006**, *29*, S21–S40. [[CrossRef](#)]
33. van Dalen, B.M.; Caliskan, K.; Soliman, O.I.; Nemes, A.; Vletter, W.B.; Ten Cate, F.J.; Geleijnse, M.L. Left ventricular solid body rotation in non-compaction cardiomyopathy: A potential new objective and quantitative functional diagnostic criterion? *Eur. J. Heart Fail.* **2008**, *10*, 1088–1093. [[CrossRef](#)] [[PubMed](#)]
34. Nawaytou, H.M.; Montero, A.E.; Yubbu, P.; Calderon-Anyosa, R.J.C.; Sato, T.; O'Connor, M.J.; Miller, K.D.; Ursell, P.C.; Hoffman, J.I.E.; Banerjee, A. A Preliminary Study of Left Ventricular Rotational Mechanics in Children with Noncompaction Cardiomyopathy: Do They Influence Ventricular Function? *J. Am. Soc. Echocardiogr.* **2018**, *31*, 951–961. [[CrossRef](#)] [[PubMed](#)]
35. Pun, R.; Silverman, N.H. Cardiac segmental analysis in left ventricular noncompaction: Experience in a pediatric population. *J. Am. Soc. Echocardiogr.* **2010**, *23*, 46–53. [[CrossRef](#)] [[PubMed](#)]



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