ORIGINAL RESEARCH

RIKADA Study Reveals Risk Factors in Pediatric Primary Cardiomyopathy

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***Background*—**Cardiomyopathies are heterogeneous diseases with clinical presentations varying from asymptomatic to life-threatening events, including severe heart failure and sudden cardiac death. The role of underlying genetic and disease-modulating factors in children and adolescents is relatively unknown. In this prospective study, in-depth phenotypic and genetic characterization of pediatric patients with primary cardiomyopathy and their first-degree family members (FMs) was performed. Outcome was assessed to identify clinical risk factors.

***Methods and Results*—**Sixty index patients with primary cardiomyopathy (median age: 7.8 years) and 124 FMs were enrolled in the RIKADA (Risk Stratification in Children and Adolescents with Primary Cardiomyopathy) study. Family screening included cardiac workup and genetic testing. Using cardiologic screening, we identified 17 FMs with cardiomyopathies and 30 FMs with suspected cardiomyopathies. Adverse events appeared in 32% of index patients and were more common in those with lower body surface area (P=0.019), increased NT-proBNP (N-terminal pro–brain natriuretic peptide; P<0.001), and left ventricular dysfunction (P<0.001) and dilatation (P=0.005). The worst prognosis was observed in dilated and restrictive cardiomyopathies. Genetic variants of interest were detected in patients (79%) and FMs (67%). In all 15 families with at least 1 FM with cardiomyopathy, we found a variant of interest in the index patient. Increased number of variants of interest per patient was associated with adverse events (P=0.021). Late gadolinium enhancement was related to positive genotypes in patients (P=0.041).

***Conclusions*—**Lower body surface area, increased NT-proBNP, left ventricular dysfunction or dilatation, late gadolinium enhancement, and increased number of variants of interest were associated with adverse outcome and should be considered for risk assessment in pediatric primary cardiomyopathies.

***Clinical Trial Registration*—**URL: https://www.clinicaltrials.gov/. Unique identifier: NCT03572569. **(*J Am Heart Assoc*. 2019;8: e012531. DOI: 10.1161/JAHA.119.012531.)**

Key Words: cardiomyopathy • heart failure • pediatrics • risk assessment

Cardiomyopathies are a heterogeneous group of myocardial diseases, especially in the pediatric population. Dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) are the most common; restrictive cardiomyopathy (RCM), left ventricular noncompaction cardiomyopathy (LVNC), and mixed cardiomyopathies occur infrequently; and arrhythmogenic right ventricular cardiomyopathy (ARVC) is rare.[1] According to the American Heart

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Accompanying Tables S1 through S5 and Figure S1 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.012531

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Clinical Perspective

What Is New?

- This prospective study highlights parameters for adverse outcome in primary pediatric cardiomyopathy over a variety of phenotypes.
- Adverse events occur in nearly a third of the index patients, and patients with dilated and restrictive cardiomyopathies have the worst prognosis.
- With systematic family screening, hypertrabeculated myocardium is the most frequently occurring cardiac abnormality found in family members.

What Are the Clinical Implications?

- Systematic phenotype and genotype characterization provides important prognostic information in children and adolescents with primary cardiomyopathy.
- Long-term multicenter studies are needed to ascertain the underlying genetic and disease-modulating factors in children and adolescents.

Association, cardiomyopathies are associated with mechanical and/or electrical dysfunction and are classified into primary (genetic, nongenetic, acquired) and secondary forms.[2] The European Society of Cardiology groups cardiomyopathies as *DCM, HCM, RCM, ARVC*, and *unclassified*, including LVNC. Further subclassification into familial/genetic and nonfamilial/nongenetic forms is proposed.[3] In the current literature, overall incidence of cardiomyopathies of 1.13 per 100 000 children is reported.[4] Severe pediatric cardiomyopathy has a peak age of diagnosis of <1 year.[4,5] Children with cardiomyopathies often present with heart failure signs, and, accordingly, therapeutic regimens are mostly symptomatic.[6–8] Mechanical circulatory support (MCS) or heart transplantation (HTx) is often required, particularly in DCM patients with progression of heart failure.[9] Due to arrhythmia, syncope, or sudden cardiac death, patients with HCM frequently receive an implantable cardioverter-defibrillator.[10]

Mutations in sarcomere genes are commonly found in adults and children with cardiomyopathies.[1,11–14] Genetic causes of DCM are the most heterogeneous, with alterations also involving desmosomal, cytoskeletal, and mitochondrial genes.[15] Results of genetic testing require careful interpretation according to the individual patient context and family history. Clinical screening for cardiomyopathies is recommended in asymptomatic, at-risk, first-degree family members (FMs). If genetic testing is positive in the index patient, cascade genetic testing of FMs is recommended for risk assessment.[16]

There is a need to characterize pediatric patients who are at risk for development of heart failure and adverse events so as to enable family counseling and to generate individual diagnostic and therapy regimens.[17] In this prospective study, systematic pheno- and genotype characterization in pediatric patients with primary cardiomyopathies and their first-degree FMs was performed with a focus on identifying clinical risk factors.

Methods

Data

To minimize the possibility of unintentionally sharing information that can be used to reidentify private information, a subset of the data generated for this study is available in the ClinVar database.[18]

Study Population

Patients aged ≤18 years with a diagnosis of primary cardiomyopathies and their first-degree FMs including siblings and parents were prospectively enrolled in the RIKADA (Risk Stratification in Children and Adolescents with Primary Cardiomyopathy) study between February 2014 and January 2017 at the Charité-Universitätsmedizin Berlin and the German Heart Center Berlin in Berlin, Germany.

The following inclusion criteria were used: DCM indicated by left ventricular (LV) systolic dysfunction and dilatation >2 SD above the mean of a normal population[19]; HCM indicated by LV hypertrophy and septal wall thickness >2 SD[10,19]; RCM indicated by diastolic dysfunction and concordant atrial enlargement[20]; LVNC shown by separation of the myocardium into compacted and noncompacted layers with a noncompacted/compacted ratio >2 at transthoracic echocardiography (TTE)[21] and/or >2.3 at cardiovascular magnetic resonance (CMR)[22]; and ARVC based on the revised task force criteria.[23]. In adults, TTE reference values for LV end-diastolic diameter, septal thickness, and ejection fraction (EF) were chosen according to Lang et al,[24] and CMR reference values for ventricular dimensions were chosen according to Hudsmith et al,[25] considering values beyond the reference limits as abnormal. The clinical diagnosis of HCM was based on the presence of otherwise unexplained increase in LV wall thickness ≥13 mm in ≥1 LV myocardial segment, as measured using TTE or CMR.[10]

Suspected cardiomyopathies in FMs were defined as follows: hypertrabeculation with a noncompacted/compacted ratio <2 in TTE and/or <2.3 in CMR, mildly reduced LV systolic function (EF 50–54%) at TTE and/or CMR, borderline or mildly increased LV end-diastolic diameter at TTE and/or LV end-diastolic volume at CMR, or mildly increased septal

thickness and/or myocardial crypts at TTE and/or CMR. Individuals with evidence of myocardial inflammation or myocarditis, systemic disease with cardiac involvement, or structural congenital heart disease were excluded. Within family screening, complete cardiac workup and genetic testing were performed in index patients and both clinically affected and unaffected FMs (Figure 1).

The study was approved by the institutional ethics committee (Charité-Universitätsmedizin Berlin) in accordance with the Declaration of Helsinki, and all participants and parents or guardians of patients <18 years gave written informed consent.

Follow-Up

The occurrence of adverse events since the date of cardiomyopathy diagnosis including MCS, HTx, and all-cause death, including sudden cardiac death, was noted from medical records and defined as a combined end point.

Laboratory and Genetic Testing

A standard laboratory testing including NT-proBNP (N-terminal pro–brain natriuretic peptide) was applied. Genetic screening was performed with next-generation sequencing for genetic variants in 174 target genes (Illumina TruSight Cardio Sequencing Panel).[26] A total of 89 cardiomyopathy genes were bioinformatically filtered with a minor allele frequency of <0.001 (gnomAD reference database, https://gnomad.broadinstitute.org/) and classified according to the guidelines of the American College of Medical Genetics and Genomics (ACMG; Table S1).[27] Each detected variant was validated (as of November 30, 2017) with databases displaying genetic variants for cardiomyopathy: Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), Exome Variant Server (http://evs.gs.washington.edu/EVS/) and the Atlas of Cardiac Genetic Variation (https://cardiodb.org/ACGV/). Genetic variants of interest (VOIs) included variants of unknown significance (VUSs), likely pathogenic and pathogenic variants. Genetic data were analyzed with regard to genotype positivity (detection of VOI), grade of pathogenicity (VUS, likely pathogenic; or pathogenic) and number of VOI (Figure S1). Patients were classified as *genetic* if they had a VOI and as *sporadic* if they had no VOI or a family history of cardiomyopathy. No VOI but positive family history of cardiomyopathy was seen in only 1 index patient who was thus excluded from the Kaplan–Meier analysis. An index patient was categorized as having *familial cardiomyopathy* if a cardiomyopathy was present in at least 1 first-degree FM. Novel VOIs will be deposited in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/).[18]

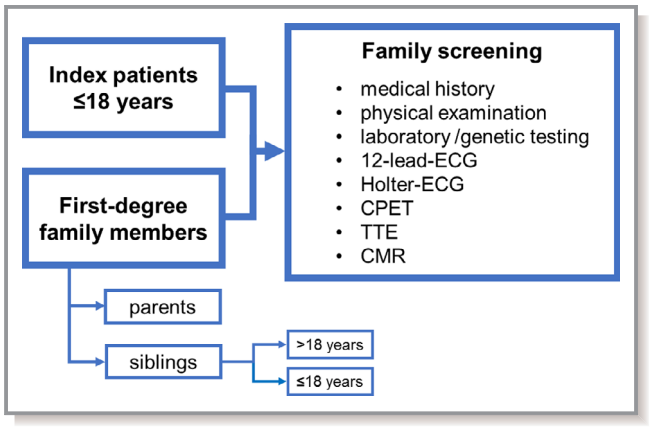

Figure 1. Study design. The protocol of the RIKADA (Risk Stratification in Children and Adolescents with Primary Cardiomyopathy) study involved in-depth family screening of 60 pediatric index patients and all available first-degree family members (FMs). CMR was performed in index patients and FMs, including siblings aged ≤18 years, for preclinical detection of cardiomyopathy. CMR indicates cardiovascular magnetic resonance; CPET, cardiopulmonary exercise testing; TTE, transthoracic echocardiography.

Cardiopulmonary Exercise Testing

Symptom-limited cardiopulmonary exercise testing under strict monitoring of signs of ischemia and arrhythmia was performed on a treadmill ergometer in adherence to the protocol of the German Society of Pediatric Cardiology, and data were compared with reference values for children and adults.[28]

Echocardiography

Detailed TTE, considering cardiac morphology, dimensions, and systolic and diastolic function, was conducted at rest, and offline analyses were carried out by 2 independent observers (N.A.M., F.D.) according to the recommendations for adults and children.[24,29]

Cardiovascular Magnetic Resonance

All CMR studies were performed at 1.5 T, using a standardized protocol with acquisition of images in short and long axis and axial orientation to allow for detailed morphologic and functional analyses and myocardial tissue characterization. Late gadolinium enhancement (LGE) images were acquired after bolus administration of gadolinium-DOTA (Dotarem; Guerbet). Siblings aged ≤18 years underwent CMR when sedation was not necessary.

Statistical Analyses

Categorical variables are summarized by frequencies and percentages. For continuous measures, data are presented as median values and interquartile ranges. The Fisher exact test

was used to compare dichotomous variables in tables with expected cell frequencies of <5; otherwise, the Pearson χ^2 test was performed. For comparison of 2 and ≥2 independent groups, the Mann-Whitney *U* test and the Kruskal-Wallis test were applied, respectively. Survival analysis was performed with the Kaplan–Meier method to estimate the probability of survival in all index patients and to compare survival distributions of different groups, defining time at diagnosis as time point 0. Overall differences between estimated survival curves of ≥2 groups were assessed with the log-rank test. The Kaplan–Meier method, a nonparametric estimator of the survival function, is widely used to estimate and graph survival probabilities as a function of time. It can be used to obtain univariate descriptive statistics for survival data, including median survival time, and compare the survival experience for ≥2 groups of participants. To test for overall differences between estimated survival curves of ≥2 groups of participants, such as between different cardiomyopathy subgroups or genetic versus sporadic cardiomyopathies, several tests are available, including the log-rank test. This can be performed as a type of χ^2 test, a method for comparing the Kaplan–Meier curves estimated for each group of participants. A probability value <0.05 is considered statistically significant. Data were analyzed with SPSS v24.0 (IBM Corp).

Results

Family Screening

Sixty index patients with a median age of 7.8 years were enrolled (35 male, 25 female; Table S2). Diagnoses included 21 DCM, 17 HCM, 15 LVNC, 5 RCM, and 2 ARVC. In total, 124 FMs were enrolled (Figure 2). This group comprised 25 siblings aged ≤18 years (median: 10.6 years), 8 siblings aged >18 years (median: 19.6 years), and 91 parents (median age: 40.0 years; Tables S3 and S4). Family screening of 60 families identified cardiomyopathies in 17 of 124 FMs. In 11 of 124 FMs, cardiomyopathies had been diagnosed before study enrollment. In addition, clinical screening in this study revealed cardiomyopathies in 6 of 124 FMs, and in 2 families, 2 FMs were affected; therefore, family screening showed cardiomyopathies in 15 of the 60 screened families (25%; Figure 2). A positive family history was present in 36% of families.

Of 57 index patients, 45 (n=79%) had at least 1 genetic VOI, and 12 index patients had none. In the index patients, 1 VOI was found in 39% and >1 VOI was found in 40%. Of all genotype-positive index patients, classification according to the highest grade of pathogenicity revealed pathogenic VOIs in 18 (40%), likely pathogenic VOIs in 16 (36%), and VUSs in

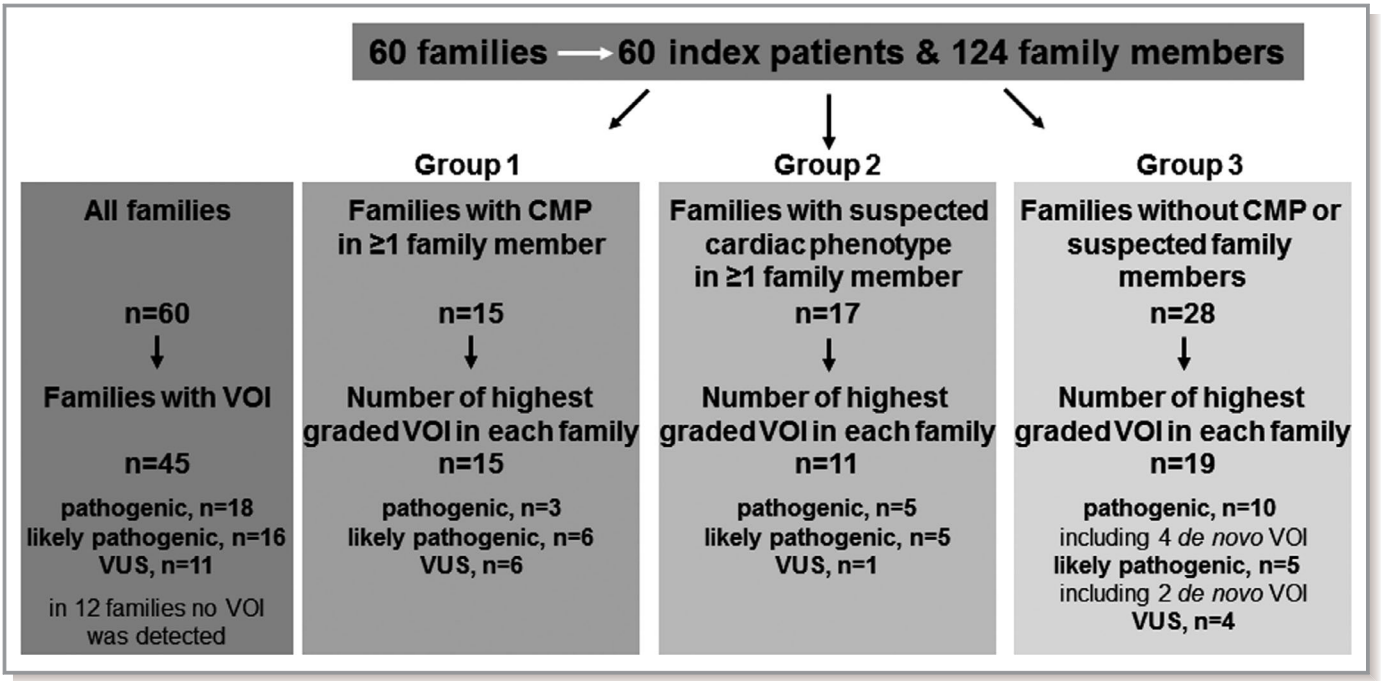

Figure 2. Clinical diagnosis and genetic information in the family context. For the RIKADA (Risk Stratification in Children and Adolescents with Primary Cardiomyopathy) study, 60 index patients and 124 family members (FMs) were enrolled. These 60 families were classified according to their clinical diagnosis of the first-degree FM either as family with cardiomyopathy (group 1), family with a suspected cardiac phenotype (group 2), or as family without detection of any cardiovascular signs (group 3). In families with a suspected cardiac phenotype, we most frequently observed left ventricular hypertrabeculation. Overall, 57 of 60 index patients underwent genetic testing. The genetic information was implemented by counting the highest graded VOIs in each family. Families with a de novo VOI were listed as families without cardiovascular signs. In 12 families, we did not detect any VOI. CMP indicates cardiomyopathy; VOI, variant of interest; VUS, variant of unknown significance.

11 (24%). DNA was not available for 3 index patients, and family screening was not performed for 2 adopted children. Consequently, 45 index patients had genetic cardiomyopathies, 10 had sporadic cardiomyopathies, and 5 remained undetermined. In 26 of 45 (58%) index patients with VOIs, family history was negative for cardiomyopathies. Only 1 patient had a positive family history with a negative genotype.

Identification of at-risk relatives with cardiologic screening

CMR images in Figure 3A highlight typical cardiomyopathy phenotypes for DCM, HCM, LVNC, and ARVC. Of the 17 FMs diagnosed with a cardiomyopathy, 4 had DCM, 5 had LVNC, 5 had HCM, and 3 had RCM. Fourteen FMs had the same phenotype as the index patient in the family.

With cardiologic screening, we identified 30 FMs with suspected cardiomyopathies. Nineteen had hypertrabeculation without LVNC (Figure 3B), 4 had mildly reduced LVEF, 3 had borderline or mildly increased LV dimensions, 3 had mildly increased interventricular septum thickness, and 1 had biventricular enlargement and hypertrabeculation. Hypertrabeculation was the most frequently found cardiac abnormality in FMs. Twenty of the 30 FMs with suspected cardiomyopathies had genetic testing and 16 had VOIs, of which 9 were likely pathogenic or pathogenic.

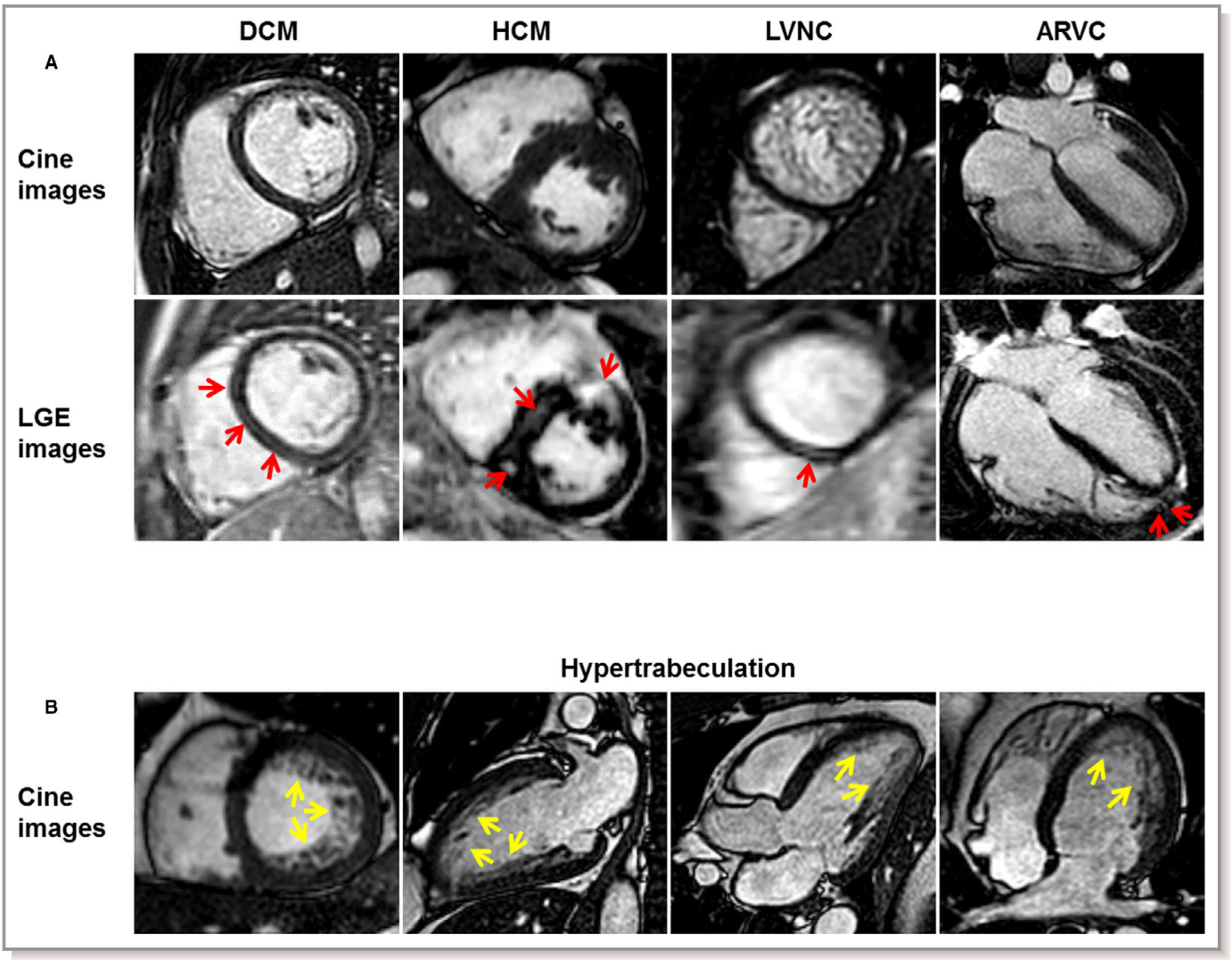

Figure 3. Cardiovascular magnetic resonance (CMR) in pediatric primary cardiomyopathy. **A**, CMR images highlight typical cardiomyopathy phenotypes for DCM, HCM, LVNC, and ARVC. Cine (upper row) and corresponding LGE (lower row) images are presented. Red arrows indicate regions with positive LGE. **B**, Hypertrabeculation is shown for individual 1-II:1 in short-axis, 2-, 3- and 4-chamber views (yellow arrows). ARVC indicates arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LGE, late gadolinium enhancement; LVNC, left ventricular noncompaction cardiomyopathy.

At least 1 FM with suspected cardiomyopathy was identified in 17 families, and 28 families were without any FM who was affected or had suspected cardiomyopathy (Figure 2). In all families with a least 1 FM with cardiomyopathy, we found a VOI in the index patient (15/15). In the 17 families including at least 1 additional FM with suspected cardiomyopathy, a VOI was found in 11 index patients. In 28 of 60 families with cardiomyopathies that had no further FM with cardiomyopathy or suspected cardiomyopathy, a VOI was present in 19 of the 28 index patients.

Genetic-variant burden in FMs

We used targeted sequencing in 45 families to test 90 FMs for the VOI previously identified in the index patient (Figure 2). At least 1 VOI was detected in 60 individuals (67%). Among the genotype-positive FMs, we identified 1 VOI in 67% and >1 VOI in 33%. Of all genotype-positive FMs, classification according to the highest grade of pathogenicity revealed the following distribution of VOIs: pathogenic in 13 of 60 (22%), likely pathogenic in 19 of 60 (32%), and VUSs in 28 of 60 (47%).

Clinical and Genetic Characteristics of Index Patients

DCM and RCM patients presented with higher New York Heart Association classes (P=0.047), increased frequencies of heart failure symptoms (P<0.001), elevated NT-proBNP levels (P=0.073), and reduced maximum oxygen consumption (P=0.337; Figure 4 and Table S2). TTE revealed the most severe LV dysfunction (P<0.001) and dilatation (P<0.001) in patients with DCM. Positive LGE was associated with a positive genotype (P=0.041). All 8 patients with positive LGE had a positive genotype.

CMR was performed in 8 HCM patients. Positive LGE at CMR was present in 4 of 8 of the HCM patients. In those, NT-proBNP levels (P<0.001) and E/E′ at TTE (P=0.019) were elevated, and LV hypertrophy was pronounced with greater interventricular septum diastolic diameter Z scores at TTE (P=0.038) and indexed LV mass at CMR (P=0.002) than in HCM patients with negative LGE.

At Holter ECG, arrhythmias including supraventricular and nonsustained ventricular tachycardias occurred with low

	Index patients					Family members		
	ALL	DCM	HCM	LVNC	RCM	Parents	Siblings >18 years	Siblings ≤18 years
Female, n	25	12	4	7	2	51	4	13
Male, n	35	9	13	8	3	40	4	12
Age, median in years (IQR)	7.8 (4–14)	6.8 (1–14)	12.1 (4–16)	7.8 (4–12)	4.2 (3–11)	40.0 (36–47)	19.6 (19–21)	10.6 (9–14)
Heart failure, n	17	9	0	4	4	0	0	2
NT-proBNP, median in pg/mL	1496 [n=53]	4947 [n=21]	1949 [n=14]	157 [n=11]	2384 [n=5]	49 [n=83]	73 [n=8]	30 [n=13]
Arrhythmias, n	7 [n=43]	4 [n=13]	1 [n=13]	1 [n=13]	1 [n=2]	6 [n=80]	0 [n=0]	0 [n=20]
LVEF, median in %	55 [n=58]	35 [n=21]	62 [n=16]	56 [n=15]	61 [n=5]	59 [n=73]	61 [n=8]	62 [n=24]
LGE (CMR), n	8 [n=28]	2 [n=11]	4 [n=8]	1 [n=6]	0 [n=2]	4 [n=60]	0 [n=6]	0 [n=9]
MACE, n	40	20	5	5	10	0	0	2

Figure 4. Clinical characterization of index patients and family members. Clinical information for all study individuals is presented in absolute values for each subgroup. Heart failure was defined as acute right or left heart failure with peripheral edema and/or pulmonary congestion. Arrhythmias included supraventricular and nonsustained ventricular tachycardia and were recorded with Holter ECG. MACE summarizes mechanical circulatory support, heart transplantation, and death. Values for arrhythmogenic right ventricular cardiomyopathy (n=2) are not presented. Of note, 4 of 8 HCM patients tested LGE positive. The full clinical information for each group is available in Tables S2 through S5. The sample size value indicates the number of analyzed individuals for a given parameter. CMR indicates cardiovascular magnetic resonance; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; IQR, interquartile range; LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; LVNC, left ventricular noncompaction cardiomyopathy; MACE, major adverse cardiovascular events; NT-proBNP, N-terminal pro–brain natriuretic peptide; RCM, restrictive cardiomyopathy.

frequency (7/43 index patients). An association between arrhythmia and LGE from CMR was not seen. Arrhythmias and signs of ischemia were not observed during cardiopulmonary exercise testing. Clinical characteristics of index patients are summarized in Table S2.

Six patients received an implantable cardioverter-defibrillator for prevention of sudden cardiac death, 5 of them for primary prevention. No appropriate implantable cardioverter-defibrillator shocks were recorded. Ten patients needed MCS with a ventricular assist device, and 3 patients received extracorporal membrane oxygenation and a ventricular assist device. Fifteen patients underwent HTx, of which 1 RCM patient had combined HTx and lung transplantation. Two children with RCM died. In one, the cause of death was sepsis with multiple organ failure; the other had sudden cardiac death 3 months after HTx (Table S3). The detection rate for >1 VOI was significantly higher in HTx patients than in non-HTx patients (P=0.011).

The clinical characteristics of FMs as part of the family screening are depicted in Figure 4 and Tables S4 and S5.

Follow-Up of Index Patients

No patient was lost to follow-up. During a median follow-up of 2.9 years (range: 1.2-6.9 years), adverse events occurred in 32% of the index patients (Table S3; Figure 5A). The combined end point was more common in children with lower body surface area (BSA; P=0.019), increased NT-proBNP (P<0.001), reduced LVEF (TTE: P<0.001; CMR: P=0.062), and LV dilatation (TTE: LV end-diastolic diameter Z scores, P=0.005; CMR: indexed LV end-diastolic volume, P=0.005).

Event-free survival was significantly different between cardiomyopathy subgroups regarding death (P<0.001), HTx (P=0.002), MCS (P=0.012), and the combined end point (P<0.001). Prognosis was worst in patients with RCM and DCM (Table S3; Figure 5B). Age at diagnosis (P=0.276) and sex (P=0.282) had no significant impact on the combined end point.

Survival analysis showed that the probability of adverse events was significantly higher in patients with >1 VOI compared with those with 1 or no VOI (P=0.021; Figure 5C). Patients with sporadic cardiomyopathies had fewer adverse events than those with genetic cardiomyopathies, albeit without a statistically significant difference (P=0.305; Figure 5D). The event-free rate for the combined end point was not significantly affected by genotype positivity (P=0.441), grade of pathogenicity (P=0.893), or family history (familial vs. nonfamilial cardiomyopathy, P=0.365).

Familial Segregation

The clinical courses of family 1 (Figure 6A) and family 2 (Figure 6B) were remarkable because of infant cardiomyopathy requiring HTx in the index patients. In both families, the severe phenotypes of LVNC and DCM, respectively, were caused by a double VOI; one was de novo, and one was inherited from a parent. In family 1, a de novo pathogenic variant in ACTN2 (α-actinin 2) produces a stop codon at amino acid position 192, which leads to premature truncation of the protein. In addition, a VUS (p.Gly142Val) in *MYLK2* (myosin light chain kinase 2) was detected in the clinically asymptomatic father and in the index patient. The father was grouped as *suspected cardiac phenotype* because of LV hypertrabeculation that did not fulfill the criteria for LVNC (individual 1-I:1; Figure 3B). In family 2, another *MYLK2*-likely pathogenic missense variant, p.Ala2Thr, was found in the index patient and his clinically asymptomatic mother, who presented with LV hypertrabeculation on CMR. A likely pathogenic *ACTC1* (α-cardiac actinin) missense variant, p.Ala110Thr, occurred de novo in the index patient of family 2.

In family 3 (Figure 6C), an X-chromosomal inheritance pattern with a likely pathogenic hemizygous variant occurred in the male index patient. He was diagnosed with LVNC as an infant and had inherited the variant in *TAZ* (taffazin), Val119Met, from his mother. Cardiologic screening of the mother (individual 3-I:1) was unremarkable except for the presence of LV hypertrabeculation on CMR. In the index patient, clinical evaluation showed no evidence of Barth syndrome but isolated LVNC.

Family 4 (Figure 6D) represents an example of a family with an index patient requiring HTx during infancy because of DCM and early detection of cardiomyopathy in a FM. Through the study, the clinically asymptomatic father (individual 4-I:1) was diagnosed with LVNC with reduced biventricular EFs on CMR (LVEF: 52%; right ventricular EF: 48%) and echocardiography (LVEF: 44%) but normal ventricular dimensions. Two likely pathogenic variants were present in the index patient and his father, in *TPM1* (α-tropomyosin), p.Glu114Gln, and in *PKP2* (plakophilin 2), p.Asn512Lys.

Two affected FMs of family 5 (Figure 6E), the 15-year-old index patient and her mother, presented with LVNC. Individual 5-II:1 was asymptomatic and diagnosed because of family screening, initiated because her mother had known symptomatic LVNC with palpitations but normal LV dimensions and LVEF. The mother and the index patient carried the likely pathogenic variant in *MYH7* (β-myosin heavy chain), P.Ala428Asp. In addition, the index patient inherited the likely pathogenic variant p.Thr602Ile in *MYBPC3* (cardiac myosin-binding protein C) from her unaffected father. Her LVEF deteriorated during the 3-year study period from 55% to 45%, which required medication with an angiotensin-converting enzyme inhibitor and a β-blocker and closer follow-up intervals.

In family 6 (Figure 6F), a pathogenic splice-site variant in *MYBPC3*, c.927-2A>G, and a likely pathogenic missense variant p.Cys598Arg in *LDB3* (LIM domain-binding 3) was identified in the 17-year-old index patient with HCM. Both

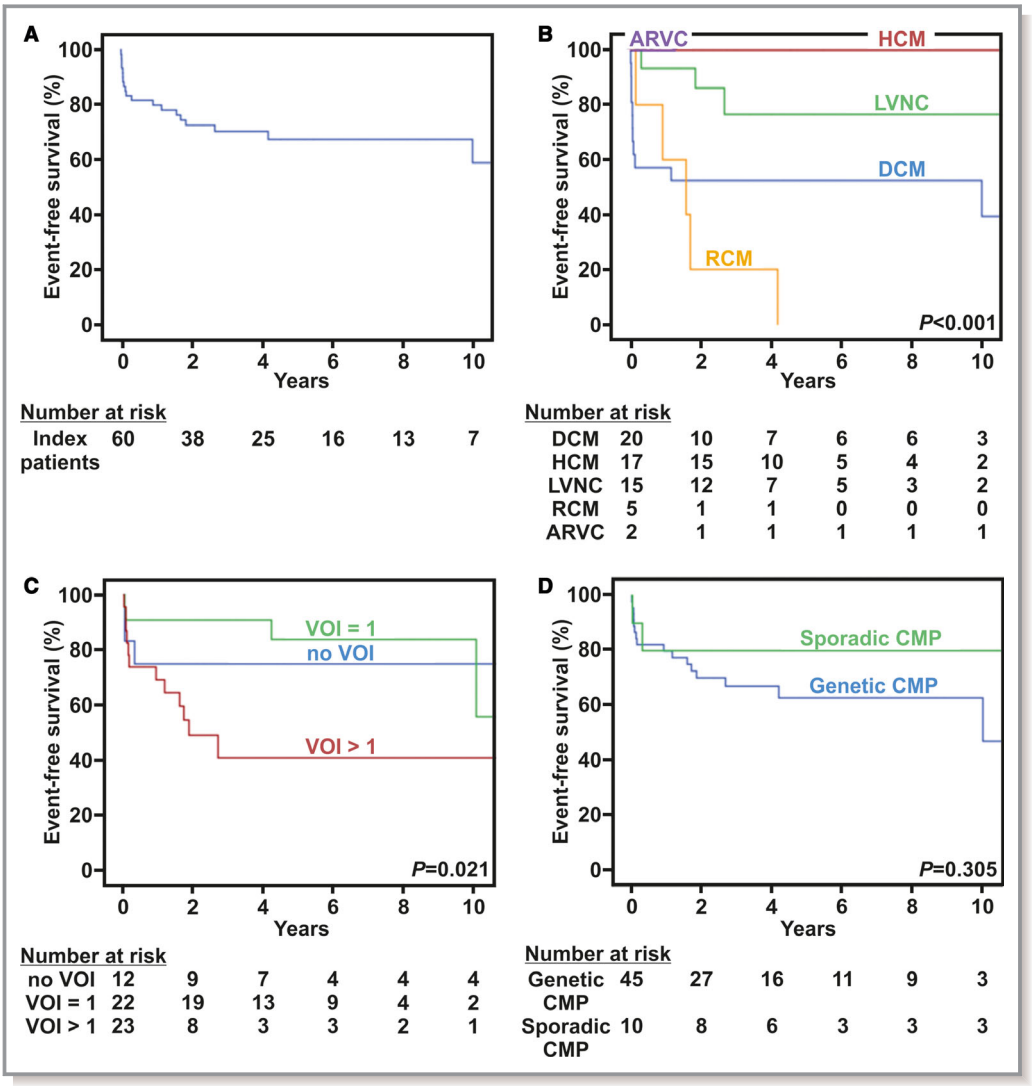

Figure 5. Adverse events in index patients with cardiomyopathy. Kaplan–Meier curves illustrate the event-free survival to the combined end point of death, heart transplantation, and mechanical circulatory support: (**A**) in all index patients, (**B**) between the different cardiomyopathy subgroups DCM, HCM, LVNC, RCM, and ARVC, (**C**) with regard to the absolute number of VOIs, and (**D**) in genetic vs sporadic cardiomyopathy. No VOI but positive family history of cardiomyopathy was seen in only 1 index patient who was thus excluded from the Kaplan–Meier analysis. ARVC indicates arrhythmogenic right ventricular cardiomyopathy; CMP, cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular noncompaction cardiomyopathy; RCM, restrictive cardiomyopathy; VOI, variant of interest.

variants were also present in his father (individual 6-I:1) and his brother (individual 6-II:3), who was 9 years old at the time of study enrollment. Individual 6-I:1 was known to have HCM and had an implantable cardioverter-defibrillator implanted at age 29. Individual 6-II:3 had extended crypts/hypertrabeculation on the initial study CMR. Individual 6-II:1, the 19-year-old sister of the index patient, was heterozygous for the MYBPC3 variant only and was suspected to have HCM with an interventricular septal thickness at end-diastole of 10 mm, but diagnostic criteria were fulfilled only by CMR with midventricular septal hypertrophy. This family exemplifies that different imaging modalities need to be combined in the evaluation of FMs for presymptomatic detection of affected status.

Discussion

Within this prospective study, we systematically analyzed the phenotypes of pediatric patients with primary cardiomyopathies and their first-degree FMs, complemented by genetic analysis. Lower BSA, increased NT-proBNP, LV dysfunction and dilatation, LGE, and increased number of VOIs were associated with adverse outcome. Adverse

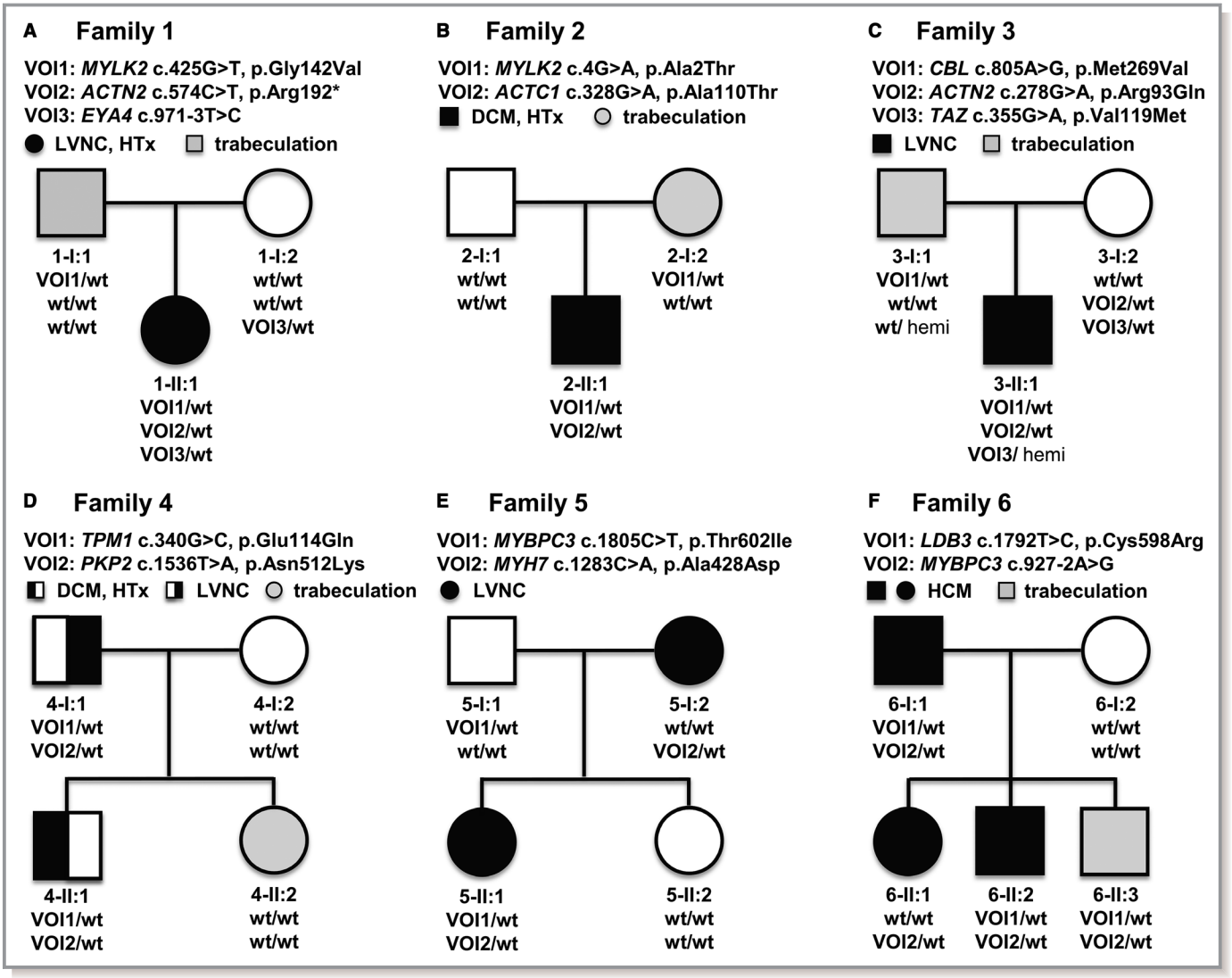

Figure 6. Pedigrees of RIKADA (Risk Stratification in Children and Adolescents with Primary Cardiomyopathy) study families. **A** through **F**, The affected individuals of the selected families demonstrate with LVNC, DCM, or HCM (black-filled symbols). Family members may present without cardiomyopathy but with a suspected cardiac phenotype (gray symbols). Detected VOIs are given for each family member. In families 1 and 2, VOI2 is a de novo variant. DCM indicates dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HTx, heart transplant; LVNC, left ventricular noncompaction cardiomyopathy; VOI, variant of interest. ACTC1, actin, alpha, cardiac muscle 1; ACTN2, actinin alpha 2; CBL, CBL proto-oncogene; EYA4, EYA transcriptional coactivator and phosphatase 4; LDB3, LIM domain binding 3; MYBPC3, myosin binding protein C, cardiac; MYH7, myosin heavy chain 7; MYLK2, myosin light chain kinase 2; PKP2, plakophilin 2; TAZ, tafazzin; TPM1, tropomyosin 1.

events occurred in nearly a third of the index patients, and DCM and RCM patients had the worst prognoses. VOIs in cardiomyopathy genes were frequently observed in both index patients and FMs (79% and 67%, respectively). Family screening suggests a large load of uncertain genetic variants in FMs or possibly nonpenetrance of pathogenic variants, which needs more long-term follow-up to verify. In 60 families, screening identified 15 families with cardiomyopathies (group 1) and 17 with a suspected cardiac phenotype (group 2). In all 15 families with an index patient and at least 1 FM with cardiomyopathy, we found a VOI in the index patient, suggesting strong penetrance of the VOI in group 1. Hypertrabeculated myocardium was the most frequently found cardiac abnormality in FMs and was observed in 20 of the 30 FMs with a suspected cardiac phenotype. In 11 of 17 FMs with a suspected cardiac phenotype, we identified a VOI. It is assumed that there is a continuum from normal, hypertrabeculated myocardium to pathological appearance of the LV myocardium.[30] We speculate that the VOIs in group 2 are less penetrant and lead to a subclinical phenotype such as hypertrabeculation. Our study supports that clinical and genetic information of index patients and FMs may predict phenotypes in relatives.[31]

Clinical signs of heart failure and imaging measures of LV dysfunction (LVEF) and dilatation (TTE: LV end-diastolic diameter *Z* scores; CMR: indexed LV end-diastolic volume) were associated with adverse outcomes. This result is in line with previous reports of children with HCM and DCM in whom congestive heart failure and severity of LV dysfunction were identified as predictors of death or HTx.[8,32] Published data on survival in pediatric cardiomyopathies have shown rather conflicting results regarding the impact of age. The above-mentioned studies on HCM and DCM also found younger age and decreased weight and body mass index to be risk factors for adverse outcome; however, in a study from the Pediatric Cardiomyopathy Registry comparing the survival of children with familial DCM with that of children with idiopathic DCM, older age was associated with an increased risk of death or HTx.[33] Alexander et al detected worse survival in DCM patients diagnosed at <4 weeks and >5 years of age.[34] In a study of pediatric HCM, the poorest prognosis was seen in patients presenting <1 year of age; however, in those surviving beyond age 1, survival was independent of the age at diagnosis.[35]

In our study, age at diagnosis had no significant impact on the combined end point, but lower BSA did. Age at diagnosis is commonly the time when clinical symptoms occur, and this can be variable in children.[4,19] Lower BSA indicated that younger children with lower weight in this study were at higher risk for adverse events.

LGE at CMR reflects focal myocardial fibrosis and has been described as a substrate for arrhythmia and adverse outcome in adult cardiomyopathies.[36] Comparatively little is known about the prevalence and clinical relevance of LGE in pediatric cohorts, and data are mainly limited to HCM.[37,38] In our study, LGE was positive in almost 30% of the index patients, with a prevalence of 50% in HCM, 18% in DCM, and 17% in LVNC patients. Comparable prevalences of 46–52% were detected in larger pediatric HCM cohorts.[37,38] We found positive LGE in the HCM subgroup to be associated with more pronounced LV hypertrophy and with clinical and imaging markers of heart failure and LV diastolic dysfunction, pointing to LGE as a risk factor in pediatric HCM. An increase of LGE over time has been described in both pediatric and adult HCM, underscoring the importance of serial CMR imaging to monitor disease progression.[38,39] In this study, arrhythmias occurred at much lower frequency than would be expected from the percentage of LGE-positive patients, especially in the HCM subgroup, with no association between arrhythmias and LGE. This may be explained by the relatively young age of the index patients, speculating that arrhythmias may occur at more advanced ages and stages of the disease. Interestingly, LGE was related to genotype positivity in index patients, which is comparable to another study.[30] Our results indicate that positive LGE in combination with positive genotype may serve for risk assessment, but the clinical importance of LGE with regard to arrhythmias in children and adolescents with primary cardiomyopathies remains to be determined.

The occurrence of adverse events was not related to sex in our index patients. However, female sex was described as an independent predictor of all-cause-mortality in adult DCM.[40] Worse survival[41] and increased disease progression risk to advanced heart failure or death[42] was also found in women with HCM. Because our pediatric patient cohort is young, one may speculate that sex-specific endocrine effects possibly affect the phenotype in adolescence or adulthood and thus may emerge at continuous follow-up.

The detection rate of >1 VOI was significantly higher in patients with HTx compared with non-HTx patients. Survival analysis showed that the probability of adverse events was significantly higher in patients with >1 VOI compared with those with 1 or no VOI. This finding agrees with reported adverse outcomes in pediatric patients with LVNC[30,43] and HCM,[44] suggesting that multiple genetic variants may act in a synergistic manner. The possible underlying mechanisms most likely depend on the individual disease genes and respective combination of variants. Whole-exome sequencing could be a useful tool to identify even more disease-associated genetic variants.[45] Limitations still exist in the interpretation of genetic variants for ACMG, and in this case, the targeted-gene panel approach was chosen.[46] Nevertheless, in this study, many VUSs were detected in the index patients (24%) and FMs (47%); this result indicates that, especially in the FMs, many VOIs may not be disease-associated or may be nonpenetrant.

A significantly higher risk of major adverse events was found in children with genetic forms of LVNC than in those with sporadic LVNC.[47] Our study consistently showed reduced event-free survival in genetic cardiomyopathies compared with sporadic forms; however, the difference did not reach statistical significance. This finding may be explained by the comparatively small sample sizes and needs to be confirmed in larger patient populations.

Limitations

The index patient cohort was heterogeneous and included different types of primary cardiomyopathies. The relatively small sizes of the cardiomyopathy subgroups limited in-depth analysis. Clinical (numeric) data of pediatric patients and adults cannot be directly compared because different reference values apply. Therefore, clinical characteristics of pediatric index patients and FMs were considered separately in the analysis. The study center offers ultimate therapy for end-stage heart failure patients, including MCS and HTx. This may have led to an overrepresentation of patients with severe, end-stage DCM in this cohort. In addition to pathogenic and

likely pathogenic variants, VUSs were also defined as VOIs. This approach may have led to overestimation of genetic versus sporadic cardiomyopathies because these variants have not yet been confirmed as disease causing and might be reclassified in the future as benign as other genomic data sets become available. Age-related disease progression is possibly not fully covered within the reported study period. Consequently, long-term follow-up examinations are required to further assess risk stratification of pediatric cardiomyopathies.

Conclusions

Systematic phenotype and genotype characterization provides important prognostic information about children and adolescents with cardiomyopathies and their first-degree FMs. Lower BSA, increased NT-proBNP, LV dysfunction and dilatation, LGE, and increased number of VOIs were associated with adverse outcome and should be used for stratified risk assessment in pediatric primary cardiomyopathies. Family screening suggests a large load of uncertain genetic variants in FMs or possibly nonpenetrance of pathogenic variants, which needs more long-term follow-up to verify.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Table S1. List of bioinformatically filtered cardiomyopathy genes.

Gene name	Gene	Protein	Chromosome	Transcript ID	Exons
ATP binding cassette subfamily C member 9	*ABCC9*	ABCC9	Chr12	ENST00000261200.8, NM_020297 ENST00000261201.8, NM_005691	38
actin, alpha 1, skeletal muscle	*ACTA1*	ACTA1	Chr1	ENST00000366684.7, NM_001100	6
actin, alpha, cardiac muscle 1	*ACTC1*	ACTC1	Chr15	ENST00000290378.4, NM_005159.4	7
actinin alpha 2	*ACTN2*	ACTN2	Chr1	ENST00000366578.5, NM_001103.2	21
Alstrom syndrome protein 1	*ALMS1*	ALMS1	Chr2	ENST00000613296.4, NM_015120.4	23
ankyrin repeat domain 1	*ANKRD1*	ANKRD1	Chr10	ENST00000371697.3, NM_014391.2	9
BCL2 associated athanogene 3	*BAG3*	BAG3	Chr10	ENST00000369085.7, NM_004281.3	4
B-Raf proto-oncogene, serine/threonine kinase	*BRAF*	BRAF	Chr7	ENST00000288602.10, NM_004333.4	18
calreticulin 3	*CALR3*	CALR3	Chr19	ENST00000269881.7, NM_145046	9
caveolin 3	*CAV3*	CAV3	Chr3	ENST00000343849.2, NM_033337	2
CBL proto-oncogene	*CBL*	CBL	Chr11	ENST00000264033.5, NM_005188.3	16
cytochrome c oxidase assembly homolog	*COX15*	COX15	Chr10	ENST00000370483.9, NM_001320975	9
crystallin alpha B	*CRYAB*	CRYAB, HSPB5	Chr11	ENST00000616970.4, NM_001885.1	4
cysteine and glycine rich protein 3	*CSRP3*	CSRP3	Chr11	ENST00000533783.1, NM_003476	7
desmin	*DES*	DES	Chr2	ENST00000373960.3, NM_001927.3	9
dystrophin	*DMD*	DMD	ChrX	ENST00000357033.8, NM_004006.2	79
DnaJ heat shock protein family (Hsp40) member C19	*DNAJC19*	DNAJC19, TIM14	Chr3	ENST00000382564.6, NM_145261	6
dolichol kinase	*DOLK*	DOLK	Chr9	ENST00000372586.3, NM_014908	1
desmocollin 2	*DSC2*	DSC2	Chr18	ENST00000280904.10, NM_024422	16
desmoglein 2	*DSG2*	DSG2	Chr18	ENST00000261590.12, NM_001943.3	15
desmoplakin	*DSP*	DSP	Chr6	ENST00000379802.7, NM_004415.2	24
dystrobrevin alpha	*DTNA*	DTNA	Chr18	ENST00000444659.5, NM_001390.4	22
emerin	*EMD*	EMD	ChrX	ENST00000369842.8, NM_000117	6
EYA transcriptional coactivator and phosphatase 4	*EYA4*	EYA4	Chr6	ENST00000355167.7, NM_172105.3	20
fibrillin 1	*FBN1*	FBN1	Chr15	ENST00000316623.9, NM_000138.4	66

four and a half LIM domains 1	*FHL1*	FHL1	ChrX	ENST00000394155.6, NM_001159702.2	8
four and a half LIM domains 2	*FHL2*	FHL2	Chr2	ENST00000344213.8, NM_201555.1	7
fukutin related protein	*FKRP*	FKRP	Chr19	ENST00000318584.9, NM_001039885	4
fukutin	*FKTN*	FKTN	Chr9	ENST00000223528.6, NM_006731	10
frataxin	*FXN*	FXN	Chr9	ENST00000377270.7, NM_000144	5
glucosidase alpha, acid	*GAA*	GAA	Chr17	ENST00000302262.7, NM_000152	20
GATA zinc finger domain containing 1	*GATAD1*	GATAD1	Chr7	ENST00000287957.3, NM_021167	5
galactosidase alpha	*GLA*	GLA	ChrX	ENST00000218516.3, NM_000169	7
hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit	*HADHA*	HADHA	Chr2	ENST00000380649.7, NM_000182	20
hyperpolarization activated cyclic nucleotide gated potassium channel 4	*HCN4*	HCN4	Chr15	ENST00000261917.3, NM_005477.2	8
hemochromatosis	*HFE*	HFE	Chr6	ENST00000357618.9, NM_000410.3	5
HRAS proto-oncogene, GTPase	*HRAS*	HRAS	Chr11	ENST00000610977.3, NM_001130442	5
heat shock protein family B (small) member 8	*HSPB8*	HSP22, HSPB8	Chr12	ENST00000281938.6, NM_014365.2	3
junctophilin-2	*JPH2*	JPH2	Chr20	ENST00000372980.3, NM_020433.4	6
junction plakoglobin	*JUP*	JUP	Chr17	ENST00000393931.7, NM_002230.2	14
KRAS proto-oncogene, GTPase	*KRAS*	KRAS	Chr12	ENST00000311936.7, NM_004985	5
laminin subunit alpha 2	*LAMA2*	LAMA2	Chr6	ENST00000421865.2, NM_000426.3	65
laminin subunit alpha 4	*LAMA4*	LAMA4	Chr6	ENST00000230538.11, NM_001105206.2	39
lysosomal associated membrane protein 2	*LAMP2*	LAMP2, CD107b	ChrX	ENST00000434600.6, NM_001122606.1	9
LIM domain binding 3	*LDB3*	LDB3	Chr10	ENST00000429277.6, NM_001171610.1	14
lamin A/C	*LMNA*	LMNA	Chr1	ENST00000368300.8, NM_170707.3	12
mitogen-activated protein kinase kinase 1	*MAP2K1*	MAP2K1, MEK1	Chr15	ENST00000307102.9, NM_002755	11
mitogen-activated protein kinase kinase 2	*MAP2K2*	MAP2K2, MEK2	Chr19	ENST00000262948.9, NM_030662	11
mindbomb E3 ubiquitin protein ligase 1	*MIB1*	MIB1	Chr18	ENST00000261537.6, NM_020774	21
myosin binding protein C, cardiac	*MYBPC3*	MYBPC3	Chr11	ENST00000545968.5, NM_000256.3	35

myosin heavy chain 6	*MYH6*	MYH6, MHC-a	Chr14	ENST00000405093.7, NM_002471.3	39
myosin heavy chain 7	*MYH7*	MYH7, MHC-b	Chr14	ENST00000355349.3, NM_000257.2	40
myosin light chain 2	*MYL2*	MYL2	Chr12	ENST00000228841.12, NM_000432.3	7
myosin light chain 3	*MYL3*	MYL3	Chr3	ENST00000292327.4, NM_000258.2	7
myosin light chain kinase 2	*MYLK2*	MYLK2	Chr20	ENST00000375985.4, NM_033118.3	13
myozenin 2	*MYOZ2*	MYOZ2	Chr4	ENST00000307128.5, NM_016599.4	6
myopalladin	*MYPN*	MYPN	Chr10	ENST00000358913.9, NM_032578	20
nexilin F-actin binding protein	*NEXN*	NEXN	Chr1	ENST00000334785.11, NM_144573.3	13
NK2 homeobox 5	*NKX2-5*	NKX2-5	Chr5	ENST00000329198.4, NM_004387.3	2
NRAS proto-oncogene, GTPase	*NRAS*	NRAS	Chr1	ENST00000369535.4, NM_002524	7
PDZ and LIM domain 3	*PDLIM3*	PDLIM3	Chr4	ENST00000284770.9, NM_014476	7
plakophilin 2	*PKP2*	PKP2	Chr12	ENST00000070846.10, NM_004572.3	14
phospholamban	*PLN*	PLN	Chr6	ENST00000357525.5, NM_002667	2
PR/SET domain 16	*PRDM16*	PRDM16, MEL1	Chr1	ENST00000270722.9, NM_022114.3	17
protein kinase AMP-activated non-catalytic subunit gamma 2	*PRKAG2*	PRKAG2	Chr7	ENST00000287878.8, NM_016203.3	16
protein tyrosine phosphatase, non-receptor type 11	*PTPN11*	PTPN11	Chr12	ENST00000635625.1, NM_001330437	15
Raf-1 proto-oncogene, serine/threonine kinase	*RAF1*	RAF1	Chr3	ENST00000251849.8, NM_002880.3	17
RNA binding motif protein 20	*RBM20*	RBM20	Chr10	ENST00000369519.3, NM_001134363.1	14
ryanodine receptor 2	*RYR2*	RYR2	Chr1	ENST00000366574.6, NM_001035.2	105
sodium channel protein type 5 subunit alpha	*SCN5A*	SCN5A	Chr3	ENST00000413689.5, NM_001099404.1	28
cytochrome c oxidase assembly protein	*SCO2*	SCO2	Chr22	ENST00000252785.3, NM_001169111	2
succinate dehydrogenase complex flavoprotein subunit A	*SDHA*	SDHA, SDH2	Chr5	ENST00000264932.10, NM_004168	15
sarcoglycan beta	*SGCB*	SGCB	Chr4	ENST00000381431.9, NM_000232.4	6
sarcoglycan delta	*SGCD*	SGCD	Chr5	ENST00000435422.7, NM_000337.5	8
sarcoglycan gamma	*SGCG*	SGCG	Chr13	ENST00000218867.3, NM_000231	8
SHOC2, leucine rich repeat scaffold protein	*SHOC2*	SHOC2	Chr10	ENST00000369452.8, NM_007373.3	9

SOS Ras/Rac guanine nucleotide exchange factor 1	*SOS1*	SOS1	Chr2	ENST00000402219.6, NM_005633.3	23
tafazzin	*TAZ*	TAZ	ChrX	ENST00000601016.5, NM_000116	11
T-box 20	*TBX20*	TBX20	Chr7	ENST00000408931.3, NM_001077653.2	8
titin-cap, telethonin	*TCAP*	TCAP	Chr17	ENST00000309889.2, NM_003673	2
transforming growth factor beta 3	*TGFB3*	TGFB3	Chr14	ENST00000238682.7, NM_003239.2	7
transmembrane protein 43	*TMEM43*	TMEM43	Chr3	ENST00000306077.4, NM_024334.2	12
troponin C1, slow skeletal and cardiac type	*TNNC1*	TNNC1	Chr3	ENST00000232975.7, NM_003280.2	6
troponin I3, cardiac type	*TNNI3*	TNNI3	Chr19	ENST00000344887.9, NM_000363.4	8
troponin T2, cardiac type	*TNNT2*	TNNT2	Chr1	ENST00000236918.11, NM_001276345	16
tropomyosin 1	*TPM1*	TPM1	Chr15	ENST00000403994.7, NM_001018005.1	9
titin	*TTN*	titin	Chr2	ENST00000589042.5, NM_001267550.1	363
transthyretin	*TTR*	TTR	Chr18	ENST00000237014.7 NM_000371.3	4
vinculin	*VCL*	VCL	Chr10	ENST00000211998.9, NM_014000.2	22

Table S2. Clinical characteristics of index patients.

	All n=60	DCM n=21	HCM n=17	LVNC n=15	RCM n=5	ARVC n=2	p-value
Female	25 (42)	12 (57)	4 (24)	7 (47)	2 (40)	0 (0)	0.215
Age (years)	7.8 (4.0-14.4)	6.8 (1.0-13.8)	12.1 (4.2-16.3)	7.8 (4.4-11.7)	4.2 (3.3-11.2)	11.7*	0.450
BSA (kg/m^2)	1.0 (0.5-1.5)	0.8 (0.3-1.5)	1.3 (0.9-1.9)	0.8 (0.4-1.2)	0.5 (0.5-1.2)	1.4*	0.108
NYHA							
I	37 (62)	8 (38)	13 (77)	12 (80)	2 (40)	2 (100)	
II	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
III	8 (13)	4 (19)	2 (12)	0 (0)	2 (40)	0 (0)	0.047
IV	2 (3)	1 (5)	0 (0)	0 (0)	1 (20)	0 (0)	
n.a.	13 (22)	8 (38)	2 (12)	3 (20)	0 (0)	0 (0)	
Heart failure signs	17 (28)	9 (43)	0 (0)	4 (27)	4 (80)	0 (0)	<0.001
Arrhythmias†							
SVT	2 (5)	1 (8)	0 (0)	0 (0)	1 (50)	0 (0)	0.594
nsVTs	5 (12) (n=43)	3 (23) (n=13)	1 (8) (n=13)	1 (8) (n=13)	0 (0) (n=2)	0 (0) (n=2)	
NT-proBNP (pg/ml)	1496 (91-9215) n=53	4947 (123-24841) n=21	1949 (130-2906) n=14	157 (69-13488) n=11	2384 (1659-11030) n=5	12* n=2	0.073
VO_2max (ml/kg*min)	33 (24-40) n=24	23 (23-40) n=5	32 (28-39) n=11	39 (27-41) n=6	13* n=1	40* n=1	0.337
Echocardiography							
Z-score LVIDD (mm)	1.6 (-1.2-4.9) n=60	5.5 (3.7-9.7) n=21	-0.9 (-3.2 - -0.1) n=17	1.6 (-0.2-4.9) n=15	-1.8 (-2.5 - -1.4) n=5	0.2* n=2	<0.001
Z-Score IVSD (mm)	0.6 (-0.4-1.9) n=59	-0.4 (-1.3-1.3) n=20	5.8 (2.7-15.5) n=17	0.2 (-0.2-1.0) n=15	-0.4 (-0.4-0.5) n=5	-0.4* n=2	<0.001
LV-EF (%)	55 (34-61) n=58	35 (17-49) n=21	62 (57-74) n=16	56 (29-58 n=15	61 (48-70) n=5	62* n=2	<0.001
E/E´	6.7 (5.1-9.5) n=42	8.4 (5.4-13.7) n=15	6.4 (4.6-12.2) n=13	7.1 (5.3-9.5) n=8	6.3 (4.3-8.1) n=4	6.1 n=2	0.819
LA (cm^2)	11.8 (7.0-15.7) n=51	12.3 (5.8-16.3) n=18	11.8 (10.7-15.2) n=16	11.2 (6.2-14.0) n=10	15.7 (9.5-21.9) n=5	9.9 n=2	0.623
CMR							
LVEDVi (ml/m^2)	95 (83-122) n=28	124 (113-224) n=11	86 (82-91) n=8	93 (80-98) n=6	87* n=2	95* n=1	0.014
LVEF (%)	57 (46-64) n=28	33 (14-52) n=11	65 (61-72) n=8	56 (53-61) n=6	77* n=2	53* n=1	0.001
LA (cm^2)	20.3 (17.6-24.7)	19.7 (16.5-26.5)	22.5 (18.8-25.1)	17.8 (17.0-20.8)	40.0	21.2	0.266

	n=26	n=10	n=8	n=6	n=1	n=1	
LGE positive	8 (29) n=28	2 (18) n=11	4 (50) n=8	1 (17) n=6	0 (0) n=2	1 (100) n=1	0.230

Values are n (%) or median (interquartile range); *only median. †Arrhythmias were recorded with Holter-ECG.

ARVC = arrhythmogenic right ventricular cardiomyopathy; BSA = body surface area; CMR = cardiovascular magnetic resonance; DCM = dilated cardiomyopathy; HCM = hypertrophic cardiomyopathy; IVSD = interventricular septum thickness at end-diastole; LA = left atrial area; LGE = late gadolinium enhancement; LVEDVi = indexed left ventricular enddiastolic volume; LVEF = left ventricular ejection fraction; LVIDD = left ventricular internal dimension at end-diastole; LVNC = left ventricular noncompaction cardiomyopathy; n.a. = not applicable; nsVT = non-sustained ventricular tachycardia; NT-proBNP = N-terminal pro brain natriuretic peptide; NYHA = New York Heart Association; RCM = restrictive cardiomyopathy; SVT = supraventricular tachycardia; VO_2max = maximum oxygen consumption.

Table S3. Devices and complications of index patients.

	All n=60	DCM n=21	HCM n=17	LVNC n=15	RCM n=5	ARVC n=2	p-value
MCS							0.004
LVAD	9 (15)	7 (33)	0 (0)	2 (13)	0 (0)	0 (0)	
BVAD	4 (7)	1 (5)	0 (0)	0 (0)	3 (60)	0 (0)	
ECMO	3 (5)	2 (10)	0 (0)	0 (0)	1 (20)	0 (0)	
ICD	6 (10)	1 (5)	5 (29)	0 (0)	0 (0)	0 (0)	0.059
HTx	16 (27)	9 (43)	0 (0)	3 (20)	4 (80)	0 (0)	<0.001
Death	2 (3)	0 (0)	0 (0)	0 (0)	2 (40)	0 (0)	0.006

Values are n (%).

ARVC = arrhythmogenic right ventricular cardiomyopathy; BVAD = biventricular assist device; DCM = dilated cardiomyopathy; ECMO = extracorporal membrane oxygenation; HCM = hypertrophic cardiomyopathy; HTx = heart transplantation; ICD = implantable cardioverter-defibrillator; LVAD = left ventricular assist device; LVNC = left ventricular noncompaction cardiomyopathy; RCM = restrictive cardiomyopathy.

Table S4. Characteristics of siblings ≤18 years of age.

	Siblings ≤ 18 years (n=25)
Female	13 (52)
Age (years)	10.6 (8.7-14.2)
BSA (kg/m^2)	1.1 (0.9-1.5)
NYHA I II III IV n.a.	 21 (91) 0 (0) 0 (0) 0 (0) 2 (9)
Heart failure signs	2 (8) n=24
Arrhythmias* SVT nsVTs	0 (0) 0 (0) n=20
NT-proBNP (pg/ml)	30 (16-79) n=13
VO_2max (ml/kg*min)	39 (32-44) n=13
Echocardiography	
Z-score LVIDD (mm)	0.1 (-0.7-1.2) n=23
Z-Score IVSD (mm)	-0.2 (-0.7-1.3) n=23
LVEF (%)	62 (56-66) n=24
E/E´	5.3 (4.6-5.9) n=14
LA (cm^2)	9.2 (6.7-11.4) n=24
CMR	
LVEDVi (ml/m^2)	86 (75-94) n=9
LVEF (%)	63 (57-65) n=9
LA (cm^2)	18.3 (13.1-21.5) n=9

LGE positive	0 (0) n=9

Values are n (%) or median (interquartile range). *Arrhythmias were recorded with Holter-ECG.

BSA = body surface area; CMR = cardiovascular magnetic resonance; IVSD = interventricular septum thickness at end-diastole; LA = left atrial area; LGE = late gadolinium enhancement; LVEDVi = indexed left ventricular enddiastolic volume; LVEF = left ventricular ejection fraction; LVIDD = left ventricular internal dimension at end-diastole; n.a. = not applicable; nsVT = non-sustained ventricular tachycardia; NT-proBNP = N-terminal pro brain natriuretic peptide; NYHA = New York Heart Association; SVT = supraventricular tachycardia; VO2max = maximum oxygen consumption.

Table S5. Characteristics of siblings >18 years of age and parents.

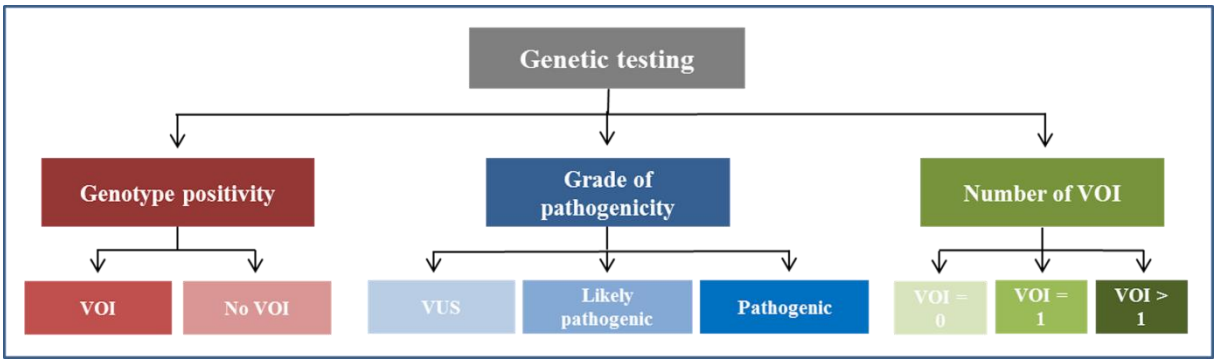
	Siblings >18 years		Parents	
	Female (n=4)	**Male (n=4)**	**Female (n=51)**	**Male (n=40)**
Age	19.1 (18.5-20.1)	20.2 (18.9-21.2)	39.3 (34.4-45.1)	43.8 (36.3-49.1)
BSA (kg/m^2)	1.8 (1.6-1.9)	2.0 (1.7-2.3)	1.79 (1.66-1.89) n=49	2.0 (1.9-2.2) n=39
NYHA				
I	4 (100)	4 (100)	48 (94)	37 (93)
II	0 (0)	0 (0)	3 (6)	3 (8)
III	0 (0)	0 (0)	0 (0)	0 (0)
IV	0 (0)	0 (0)	0 (0)	0 (0)
Arrhythmias†				
SVT	0 (0)	0 (0)	1 (2)	1 (3)
nsVT	0 (0)	0 (0)	2 (5) n=44	2 (6) n=36
NT-proBNP (pg/ml)	88 (43-104)	36 (10.4-125.4)	72 (27-140) n=47	38 (20-76) n=36
VO_2max (ml/kg*min)	31 (30-35)	33* n=3	28 (24-32) n=36	29 (24-34) n=32
Echocardiography				
LVIDD (mm)	43 (40-46)	44 (37-50)	45 (42-48) n=38	52 (46-56) n=32
IVSD (mm)	9 (7-10)	9 (8-12)	9 (7-9) n=38	10 (8-12) n=34
LVEF (%)	65 (60-68)	60 (56-65)	59 (55-62) n=40	60 (57-62) n=33
E/E´	4.6 n=3	4.3 n=3	5.3 (4.8-6.6) n=29	5.8 (4.7-7.6) n=26
LA (cm^2)	13.2 n=3	12.0 (10.2-20.1) n=4	12.1 (10.3-13.4) n=40	12.7 (10.7-17.3) n=34
CMR				
LVEDVi (ml/m^2)	79 (74-92)	84* n=2	76 (69-84) n=36	85 (77-98) n=29
LVEF (%)	64 (60-64	66* n=2	62 (60-65) n=36	61 (56-65) n=29
LA (cm^2)	18.1 (17.0-23.6) n=4	19.0 n=2	20.2 (17.2-23.0) n=36	22.0 (20.0-26.6) n=29
LGE positive	0 (0)	0 (0) n=2	2 (6) n=33	2 (7) n=27

Values are n (%) or median (interquartile range); *only median. †Arrhythmias were recorded with Holter-ECG.

Echocardiographic and CMR data are given for females and males to account for sex-specific reference values.

BSA = body surface area; CMR = cardiovascular magnetic resonance; IVSD = interventricular septum diastolic diameter; LA = left atrial area; LGE = late gadolinium enhancement; LVEDVi = indexed left ventricular enddiastolic volume; LVEF = left ventricular ejection fraction; LVIDD = left ventricular internal dimension at end-diastole; nsVT = non-sustained ventricular tachycardia; NT proBNP = N terminal pro brain natriuretic peptide; NYHA = New York Heart Association; SVT = supraventricular tachycardia; VO2max = maximum oxygen consumption.

Figure S1. Classification of genetic results.



Data were analysed according to genotype positivity, grade of pathogenicity and number of variants of interest (VOI). VUS = variants of unknown significance.