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DISSERTATION

Patients with Left Ventricular Noncompaction Cardiomyopathy - Comparison between Pediatric and Adult Phenotypes

Patienten mit linksventrikulärer Noncompaction Kardiomyopathie - Vergleich des Phänotyps zwischen Erwachsenen und Kindern

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Abbreviations

BSA - Body surface area

CMR - Cardiac magnetic resonance

DCM - Dilated cardiomyopathy

HCM - Hypertrophic cardiomyopathy

HR - Hazard ratio

HTx - Heart transplantation

ICD - Implantable cardioverter defibrillator

LGE - Late gadolinium enhancement

LP - Likely pathogenic

LV - Left ventricular

LV-EF - Left ventricular ejection fraction

LVEDD - Left ventricular end-diastolic diameter

LVNC - Left ventricular noncompaction

MACE - Major adverse cardiac event

MAF - Minor allele frequency

NGS - Next-generation sequencing

OR - Odds ratio

P - Pathogenic

VUS - Variant of uncertain significance

Abstract

Background: Left ventricular noncompaction (LVNC) is a genetically determined cardiomyopathy affecting children and adults. Current guidelines recommend genetic testing for all patients with LVNC, although defined genetic factors predicting the course of disease and phenotype are mainly unknown. Additionally, the identification of specific phenotypes increasing the risk for poor outcome in pediatric and adult patients is needed.

Methods: In this retrospective, multicenter study next-generation sequencing for 174 target cardiac disease genes was performed in unrelated adult and pediatric index patients. Family members of index patients were tested for the respective variants of the index patient with Sanger sequencing. For index patients and all their available family members clinical data was collected. Genetic and clinical data was statistically analyzed using SPSS v.26 (IBM Corporation). The main endpoint for survival analysis was major adverse cardiac events (MACE).

Results: Our cohort contained 113 unrelated index patients and 36 family members with LVNC. 40% (55/137) of patients were under the age of 18 years at diagnosis. MACE occurred in 22% (12/55) of children and 18% (15/82) of adults.

48 variants were identified in 74% (32/43) of pediatric patients, and 82 variants in 79% (52/66) of adult patients. Most frequently, variants occurred in sarcomere genes. Neither in pediatric nor adult patients did the genotype impact the risk for MACE or predict the phenotype. 62% (37/60) of family members without LVNC carried at least one variant of the respective index patient.

In the pediatric and adult cohort of LVNC patients, reduced left ventricular (LV) systolic function is the primary independent risk factor for MACE. In children, other relevant factors included increased left ventricular end-diastolic diameter (LVEDD) and lower body surface area. In adults, increased LVEDD and higher age at diagnosis were of relevance. Symptomatic patients were also at higher risk for an adverse outcome.

Conclusions: Reduced LV systolic function was identified as the most important high-risk phenotype likewise in pediatric and adult patients. The genotype did not predict the clinical outcome or phenotype.

Zusammenfassung

Hintergrund: Die linksventrikuläre Noncompaction (LVNC) ist eine seltene, genetisch bedingte Kardiomyopathie, die bei Kindern und Erwachsenen vorkommt. Genetische und klinische Faktoren, die den Krankheitsverlauf vorhersagen und Genotyp-Phänotyp-Korrelationen sind bisher größtenteils ungeklärt.

Methodik: In dieser multizentrischen, retrospektiven Studie wurden bei nicht-verwandeten Indexpatienten Next Generation Sequencing für 174 Kardiomyopathie-Gene mit dem Illumina TruSight Cardio Sequencing Panel durchgeführt. Klinische Daten wurden für die Indexpatienten und alle verfügbaren Familienmitglieder erhoben. Mithilfe von Sanger-Sequenzierung wurden die Familienmitglieder auf die jeweiligen genetischen Varianten des Indexpatienten getestet.

Ergebnisse: Unsere Kohorte bestand aus 113 nicht-verwandten Indexpatienten und 36 Familienmitgliedern mit LVNC. 40% (55/137) der Patienten waren zum Zeitpunkt der Diagnose unter 18 Jahren alt. Major adverse cardiac events (MACE) wurden in 22% (12/55) der Kinder und 18% (15/82) der Erwachsenen beobachtet. In 46% (65/143) der Patienten war eine eingeschränkte linksventrikuläre Funktion vorhanden. Risikofaktoren für kürzeres ereignisfreies Überleben in der Kaplan-Meier Analyse waren ein erhöhter linksventrikulärer enddiastolischer Diameter (LVEDD) und eine reduzierte linksventrikuläre systolische Funktion. Die reduzierte linksventrikuläre systolische Funktion war der wichtigste Risikofaktor für MACE in der multivariaten Analyse. In Kindern erhöhten geringere Körperoberfläche, erhöhter LVEDD und geringere linksventrikuläre Ejektionsfraktion das Risiko für MACE.

Insgesamt wurden 134 Varianten in 46 verschiedenen Genen in 77% (87/113) der Indexpatienten gefunden. 41 Varianten wurden als (wahrscheinlich) pathogen klassifiziert. Die meisten Varianten wurden in *MYH7* und *TTN* gefunden. 42,5% der Varianten befanden sich in Genen, die für Komponenten des Sarkomers kodieren. Das Vorhandensein von Varianten korreliert weder bei Kindern noch bei Erwachsenen mit dem Outcome, ereignisfreien Überleben oder Phänotyp. Bei 62% (37/60) der Familienmitglieder, die keine LVNC aufwiesen, konnte mindestens eine Variante des Indexpatienten nachgewiesen werden.

Schlussfolgerungen: Der größte Risikofaktor für die Vorhersage des Outcomes bei Kindern und Erwachsenen mit LVNC ist die linksventrikuläre systolische Funktion. Das ereignisfreie Überlegen wird verkürzt durch einen erhöhten LVEDD und eine reduzierte

linksventrikuläre systolische Funktion. Der Genotyp beeinflusst das Risiko für MACE und den Phänotyp nicht.

1 Introduction

1.1 Left ventricular noncompaction cardiomyopathy

Left ventricular noncompaction (LVNC) is a rare genetically determined cardiomyopathy first described in 1990 (1). LVNC can be found prenatally, in newborns, children, and adults (2-5). The European Society of Cardiology characterizes LVNC, together with Takotsubo cardiomyopathy, as an unclassified cardiomyopathy (6). The American Heart Association classifies LVNC together with hypertrophic cardiomyopathy (HCM) and arrhythmogenic right ventricular cardiomyopathy as a genetic cardiomyopathy (7).

LVNC is characterized by massive trabeculations and deep intertrabecular recesses in the left ventricle. Mandatory for the diagnosis is a two-layered myocardium with a thicker inner noncompacted and a thinner outer compacted layer (8). Often the term isolated LVNC is used. Non-isolated LVNC is defined as LVNC with additional congenital heart defects (8).

A noncompacted phenotype is also found in healthy individuals without functional impairment or other cardiac findings. Reports of a noncompacted myocardium exist, for example for women during pregnancy, athletes, black individuals, and patients with sickle cell anemia (9-13). This morphologic finding of LVNC appears without major clinical impact in these individuals, as athletes meeting the criteria for LVNC had no adverse events over four years of follow-up (11).

There has been an increased interest in distinguishing high-risk patients from those with an expected good outcome. Many recent publications have tried to specify risk factors (5, 14-16).

1.1.1 Epidemiology

It is a rare disease with an incidence of around 0,05% in adults and around 0.11 per 100.000 in children (17, 18). In the EORP Cardiomyopathy registry, LVNC was observed in 4.1% of adults and therefore the fourth most common cardiomyopathy (19). Pediatric LVNC is the third most common primary cardiomyopathy, making up 5% of cases in children under 18 years and 9.2% in children under 10 years, respectively (18, 20). Many children are diagnosed under the age of one year (18).

1.1.2 Clinical characteristics

LVNC is usually diagnosed by echocardiography or cardiac magnetic resonance (CMR) imaging. Over the years, different diagnostic criteria have been developed. Jenni et al. developed criteria for diagnosis via echocardiography based on the end-systolic ratio of noncompacted to compacted layer of the myocardium (8). Currently, the Jenni criteria and the criteria by Stollberger et al. are often used simultaneously (21). For CMR imaging, the criteria by Petersen et al. have prevailed (22). As described, the diagnosis is made by morphological parameters. Functional parameters are currently not part of the diagnostic criteria.

Patients of all ages range from asymptomatic to symptoms of decreased left ventricular (LV) systolic function, ventricular arrhythmias, and systemic emboli (1). The most common complications in adults are heart failure hospitalization and cardiac device implantation (16). Later heart transplantation (HTx) can be required (16). Due to the occurrence of arrhythmias and sudden cardiac death, an implantable cardioverter-defibrillator is necessary in some cases (16).

The survival of patients with LVNC is reduced compared to the normal population (23). The mortality in adults is 1.92 per 100 person-years in adults (16). In pediatric LVNC, the incidence of death or HTx is 18% (24). Comparing LVNC to other cardiomyopathies, a higher rate of cardiovascular events and heart failure hospitalization, and lower event-free survival in LVNC than in dilated cardiomyopathy (DCM) is reported (16, 25).

Further research is needed for better individual risk stratification and planning of therapeutic regimes. Specific risk factors must be defined to identify high-risk individuals as early as possible in this heterogeneous disease.

Many currently available studies have limitations, as they often include small, heterogenous cohorts from single centers, patients are highly selected with a severe phenotype because they were referred to a tertiary center, and different diagnostic criteria are being used.

1.1.3 Subtypes

LVNC has been classified into subgroups by different authors in the past. Towbin et al. differentiate 7 phenotypes, Jefferies et al. 5 phenotypes, and Van Waning et al. 3 phenotypes (Table 1) (18, 26, 27). All three classifications have in common that the

subtypes ‘LVNC with dilatation’ and ‘LVNC with hypertrophy’ are part of the descriptions. Van Waning et al. use the abbreviation NCCM instead of LVNC (27).

Table 1. LVNC-subtypes

(Own illustration: Schultze-Berndt)

Towbin et al. (26)	Jefferies et al. (18)	Van Waning et al. (27)
Benign LVNC	Isolated LVNC	Isolated NCCM
Dilated LVNC	Dilated LVNC	NCCM with DCM
Hypertrophic LVNC	Hypertrophic LVNC	NCCM with HCM
Hypertrophic dilated LVNC	Restrictive LVNC	
Restrictive LVNC	Indeterminate LVNC	
LVNC with arrhythmias		
Right ventricular or biventricular LVNC		
LVNC with congenital heart disease		

1.1.4 Associated genes

LVNC is considered a genetic cardiomyopathy. In a statement by the American Heart Association, for LVNC, like for all cardiomyopathies, the recording of detailed family history and clinical screening of first-degree relatives at risk is recommended (28). Genetic testing should be offered to all patients, especially smaller children and newborns. It is recommended to use a gene panel of associated cardiomyopathies for genetic testing according to the individual phenotype (e.g., DCM or HCM) (28). For isolated LVNC, no specific panel is listed (28).

A genetic cause of LVNC was reported in about 50% of patients (5). Pathogenic variants were found in 38% of patients (29). Most of the genes in which mutations were found are also associated with other primary cardiomyopathies like DCM and HCM (30). Only present in individuals with LVNC are truncating variants in *ACTN2*, *MYH7*, and *PRDM16* (30). In 5-10% of cases the appearance of LVNC might be explained by these variants (30). Sarcomere genes are most frequently affected with approximately 70% of cases of LVNC (29, 31). Most variants were found in the genes *myosin heavy chain 7* (*MYH7*), *myosin binding protein C, cardiac* (*MYBPC3*), *titin* (*TTN*), and *lamin A/C* (*LMNA*) (5, 25). The inheritance mode is autosomal dominant with a variable penetrance for most variants (32).

Although genetic testing for patients with LVNC is recommended in current guidelines, the specific consequences of these tests on treatment or prognosis in the individual

patients are mostly not known (33). Some reports exist for specific genes associated with a worse outcome.

1.2 Aims of this work

This study attempts to identify genotype-phenotype correlations and clinical and genetic risk factors for adverse outcome in 137 patients with LVNC. We analyzed clinical data retrospectively and performed genetic testing in index patients and their family members. Through family screening, we identified family members carrying variants with and without LVNC. We specifically analyzed the impact of various phenotypes of LVNC on the outcome of the pediatric and adult patients.

2 Methods

2.1 Study population

This retrospective study cohort included unrelated index patients with a diagnosis of LVNC from Charité - Universitätsmedizin Berlin, Germany, German Heart Center Berlin, Germany, University Children's Hospital Zurich, Switzerland, and University Hospital Zurich, Switzerland. The clinical data were collected retrospectively through medical records. The patients were diagnosed with LVNC between 1987 and 2017. Following the Declaration of Helsinki, the institutional ethics committees approved the study. Written informed consent was given by all participants and legal guardians of participants under the age of 18 years.

2.2 Diagnostic criteria

Experienced physicians diagnosed LVNC via echocardiography according to the gold standard by Jenni et al. (8).

Hypertrabeculated myocardium was defined as the existence of cardiac hypertrabeculation without the diagnostic criteria for LVNC being met. Patients were classified as pediatric when they were <18 years of age at the point of diagnosis. The Mosteller method was used to determine the body surface area (BSA) (34). “Symptomatic/Symptoms” was defined as the occurrence of dyspnea, palpitations, syncope, or shock. Arrhythmias were recorded by 12-lead ECG or Holter-ECG and included atrial fibrillation, non-sustained and sustained supraventricular tachycardia, atrioventricular block II°, atrioventricular block III°, and non-sustained and sustained ventricular tachycardia. A left-ventricular ejection fraction (LV-EF) <45%, or a fractional shortening <19% in men or <21% in women, respectively, was defined as reduced LV systolic function (35).

According to their phenotypic characteristics, patients were classified into three subtypes: Isolated LVNC, dilated LVNC, and hypertrophic LVNC. Dilated LVNC was defined by an increased left ventricular end-diastolic diameter (LVEDD). Hypertrophic LVNC was diagnosed when patients showed an increased LV wall-thickness. When LVEDD and LV wall-thickness both were increased, the patient was categorized as hypertrophic LVNC. When neither LVEDD nor LV wall-thickness was increased, patients were classified as isolated LVNC CMP. Patients whose values for LVEDD or LV wall thickness were unavailable were not included in the subtype analysis.

An increased LVEDD was defined as ≥54 mm in females and ≥60 mm in males (35). The threshold for LV wall-thickness was ≥13 mm (36). The echocardiographic parameters LVEDD and LV-wall thickness in pediatric patients >2 standard deviations from a normal population were used as threshold values (37).
Major adverse cardiac events (MACE) summarize mechanical circulatory support, HTx, survived sudden cardiac death, and all-cause death. MACE were used as the combined endpoint for the analysis of follow-up.

2.3 Genetic testing

All 113 index patients underwent genetic testing via next-generation sequencing (NGS) as previously described in the publication related to this thesis (38). In short, 174 cardiac disease genes were sequenced through the Illumina TruSight Cardio Sequencing Panel. As published by Kühnisch et al., 89/174 cardiomyopathy genes were bioinformatically filtered (38). A minor allele frequency (MAF) of <0.0001 (gnomAD reference database, https://gnomad.broadinstitute.org/) was used. The American College of Medical Genetics and Genomics' guidelines for classification of variants were applied (39). Variants were classified as pathogenic, likely pathogenic, and variants of uncertain significance (VUS). As previously published by Kühnisch et al., the genes were sorted into functional groups (38).
146 family members were tested for the specific variants of their index patient via Sanger sequencing.

2.3.1 Sanger sequencing

Sanger sequencing was used to verify the results generated through next-generation sequencing and to test the segregation of variants within the families. The following primers, devices, and chemicals were used for Sanger sequencing (Table 2-3).
Family members carrying at least one of their index patients variant were classified as variant carriers.

Table 2. List of primers used for Sanger sequencing

(Own illustration: Schultze-Berndt)

Gene	Primer	Primer sequence	Lenght
GAA	ghGAA_ex14-15_f	gggctctgggtcacttgg	18
GAA	ghGAA_ex14-15_r	atgttgtctcactcagcggc	20

Gene	Primer	Primer sequence	Lenght
HCN4	ghHCN4_ex4_f	aacagcaaggttagggagcc	20
HCN4	ghHCN4_ex4_r	ctgctcttccctcacactgg	20
JUP	ghJUP_ex10_f	gttcattcggctgtcatggg	20
JUP	ghJUP_ex10_r	ctcctaaccttgccctttaagc	22
KCNA5	ghKCNA5_ex1_fend	TTCTCTAGCATCCCTGACGC	20
KCNA5	ghKCNA5_ex1_rend	AGGGAGGAAAGGAGTGAAAGG	21
MYBPC3	ghMYBPC3_ex1_f	aggtggctggacaggagg	18
MYBPC3	ghMYBPC3_ex1_r	cttgctgtggaaggtgaagg	20
MYBPC3	ghMYBPC3_ex16-17_f	tgggacctgaggatgtggg	19
MYBPC3	ghMYBPC3_ex16-17_r	tgaggtttaggctgtcaaagg	21
MYH6	ghMYH6_ex26_f	ggtctgagatgcccttggg	19
MYH6	ghMYH6_ex26_r	acagagagagaaggcatggg	20
MYH7	ghMYH7_ex7-8-9_f	gaaacatttcccattcttcc	20
MYH7	ghMYH7_ex7-8-9_r	aatggagaaagatgcagagg	20
MYH7	ghMYH7_ex22_f	tttcccgcttctctgaggc	19
MYH7	ghMYH7_ex22_r	agaagtgttgatcccagagtcc	22
MYH7	ghMYH7_ex22_f2	cagcactcctttcaatgggc	20
MYH7	ghMYH7_ex22_r2	aggaacaagacagtgagccc	20
MYH7	ghMYH7_ex23-24_f	cccagtgttcccaagttatac	21
MYH7	ghMYH7_ex23-24_r	gaattgatcaccacctctga	20
MYH7	ghMYH7_ex37_f	CAGACTGAAGTGGAGGAGGC	20
MYH7	ghMYH7_ex37_r	agtgggtgttgagatggagc	20
MYLK2	ghMYLK2_ex13_f	caatacagtgtcatggcgcc	20
MYLK2	ghMYLK2_ex13_r	TTCAAAGACGAGGAGCTGGG	20
MYLK2	ghMYLK2_ex13_f2	tctctagcctgtgaccctcc	20
MYLK2	ghMYLK2_ex13_r2	ACCACAGAAGCCCTACATGC	20
MYLK2	ghMYLK2_ex13_r3	GATCCAGGCCACACTCCG	18
MYLK2	ghMYLK2_ex3_f	gtggcagctcatctctaggg	20
MYLK2	ghMYLK2_ex3_r	gcggtagaggcaattcacag	20
NEXN	ghNEXN_ex13_f	tctttcaagtcactggaatgtactg	25
NEXN	ghNEXN_ex13_r	GAGAAAGTTCCAGGGAGAGGG	21
NKX2-5	ghNKX2-5_ex1_f	CCAATGGCAGGCTGAGTCC	19
NKX2-5	ghNKX2-5_ex1_r	aggcatcttacattctgaaccc	22
NRAS	ghNRAS_ex4_f	tcctgaccttgtgatctgcc	20
NRAS	ghNRAS_ex4_r	GAATCCCGTAACTCTTGGCC	20

Gene	Primer	Primer sequence	Lenght
NRAS	ghNRAS_ex4_f2	ttgtagagaccgggttttgc	20
NRAS	ghNRAS_ex4_r2	ctgaaagctgtaccatacctgt	22
RBM20	ghRBM20_ex9_f2	ATGATCGCAAACACCACCC	19
RBM20	ghRBM20_ex9_r2	cctagcgcatagtaaatagccag	23
RBM20	ghRBM20_ex13_f	gctcagtaaccagccaagg	19
RBM20	ghRBM20_ex13_r	agactcagaggcaagcaagg	20
TBX20	ghTBX20_ex6_f	ggccttcccttaccatccc	19
TBX20	ghTBX20_ex6_r	cttcttagaggtcctgaggcc	21
TBX20	ghTBX20_ex7_f	tcagtcatgtttgcttgcagtc	22
TBX20	ghTBX20_ex7_r	actcctgatccctgactcaaag	22
TMEM43	ghTMEM43_ex8_f	ctctgacttggtgggagagg	20
TMEM43	ghTMEM43_ex8_r	ctcaggctcttctcccacc	19
TMEM43	ghTMEM43_ex8_f2	gagagggcacagggaaagc	19
TMEM43	ghTMEM43_ex8_r2	gggtggaagagaagcaggg	19
TNNC1	ghTNNC1_ex4_f	ttagcctatccgagccttgg	20
TNNC1	ghTNNC1_ex4_r	agggacactgggagatggg	19
TPM1	ghTPM1_ex6_f	gcagcccttcgtctctagg	19
TPM1	ghTPM1_ex6_r	catgcaaagaactcgccagc	20
TTN	ghTTN_ex5_f	agctggtttgtgtctaaatggg	22
TTN	ghTTN_ex5_r	GATCTGGCATCAAAGTGGGC	20
TTN	ghTTN_ex306_f1	ATGAGTTCAGGGTGTGTGCC	20
TTN	ghTTN_ex306_r1	TGTCTGCCTCACGTTTCTCC	20
TTN	ghTTN_ex326_f2	TTACCGGCTTGTCTGAAGGG	20
TTN	ghTTN_ex326_r2	AATGGTTGAAGTCGCTGTGG	20
TTN	ghTTN_in347_f	ACTGGATATGTTCTCGAGGCC	21
TTN	ghTTN_in347_r	ATGGTTTCTGAAGTAGTTCCGG	22

Table 3. Devices and materials used for Sanger sequencing

(Own illustration: Schultze-Berndt)

Device/Chemical/Enzyme/Kit	Company	Ref
DNA Engine Tetrad 2, Peltier Thermal Cycler	Bio-Rad	
NanoDrop ND-1000 Spectrophotometer	Thermo Fisher Scientific	
3730xl DNA Analyzer	Applied Biosystems	
BigDye Terminator v3.1	Thermo Fisher Scientific	4337455
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D4540

dNTP set	Rapidozym	GEN-009-250
Ethidium bromide, 1% in H2O	SIGMA-ALDRICH	46067-50ML-F
Exonuclease I	New England Biolabs	M0293
FIREPol DNA polymerase	SOLIS BIODYNE	04-11-00115
GeneRuler 100bp DNA ladder	Thermo Fisher Scientific	SM0242
Hi-Di Formamide	Thermo Fisher Scientific	4311320
Illustra Sephadex G-50 DNA Grade	GE Healthcare Life Sciences	17-0573-02
LE Agarose	Biozyme	840004
Phusion High-Fidelity DNA Polymerase	New England Biolabs Inc.	M0530
rAPid Alkaline Phosphatase	Roche/MERCK	4898141001
Taq DNA polymerase	Qiagen	201205
TERMIPol DNA polymerase	SOLIS BIODYNE	01-03-00500

2.3.2 DNA Extraction

DNA was isolated from the blood of index patients and their family members. We used the kit “NucleoSpin Blood“ from MACHEREY-NAGEL (Ref: 740951).

2.3.3 DNA Purification

DNA was purified using the kit “Invisorb® Spin DNA Extraction Kit” by STRATEC Molecular (Ref: 1050100x00).

2.4 Statistical analysis

SPSS v.26 (IBM Corporation) was used to conduct the statistical analysis. The Pearson x^2 test was used for categorical data, and in the case of a cell frequency <5, we used the Fisher exact test. The Mann-Whitney U test was used to compare continuous data for 2 independent groups. The Kruskal-Wallis test was applied to continuous data for more than two independent groups. For event-free survival analysis, Kaplan-Meier curves were used. Event-free survival was defined as time to the combined endpoint of mechanical circulatory support, HTx, survived sudden cardiac death, and all-cause death. Point zero was defined as the time of diagnosis. Patients were censored at the date of last follow-up. For comparison of the Kaplan-Meier curves, the log-rank test was used. We performed binary logistic regression for Odds ratios (OR), and for Hazard ratios (HR) Cox regression analysis.

3 Results

3.1 Cohort

3.1.1 Clinical characteristics

We included 149 individuals with a diagnosis of LVNC in our analysis. Out of those patients with LVNC, 113 were unrelated index patients, and 36 were family members of those patients. Overall, we included 202 family members from 54 families, out of which we had clinical data available from 96 individuals.

In the 149 patients with LVNC, the median age was 27.8 (9.2-44.8) years at point of diagnosis. 39% (43/109) of index patients, 43% (12/28) affected family members and 40% (55/137) of all LVNC patients were under the age of 18 years at diagnosis. Symptoms occurred in 58 % (76/132) of patients. 46% (65/143) showed a reduced LV systolic function, with the median LF-EF being 47.6% (33.0%-62.5%). We identified an increased LVEDD in 55/122 (37%) of patients, and arrhythmias occurred in 27/149 (18%). “Other CHD” included atrial septal defects and bicuspid aortic valve (Table 4). Over a median follow-up time of 5.6 (1.7-11.4) years, we have seen 36 events classified as MACE in 27/149 (18%) patients. We observed 14 heart transplantations, 11 deaths, 8 implantations of mechanical circulatory support devices and 3 survived sudden cardiac deaths over a median follow-up time of 5.6 (1.8-11.4) years (Table 4). In 3 patients, a MACE was the first symptom leading to the diagnosis of LVNC.

3.1.2 Impact of clinical factors on outcome

Symptomatic patients were at higher risk for MACE than asymptomatic patients (HR: 4.83; CI 95%: 1.43-16.33; p-Value: 0.011). A lower BSA was also associated with a higher risk for MACE (HR: 0.51; CI 95%: 0.27-0.97; p-Value 0.039). No significant effect on the risk for MACE was seen for arrhythmias (HR: 2.03; CI 95%: 0.86-4.79; p-Value: 0.108), and female gender (HR: 1.06; CI 95%: 0.45-2.50, p-Value: 0.901). In the adult cohort, higher age was associated with a higher risk for MACE (HR: 1.04; CI 95%: 1.01-1.08; p-Value: 0.019).

Echocardiographic parameters

Especially a reduced LV systolic function and an increased LVEDD showed to have a significant impact on the outcome of patients. For the appearance of MACE, we have seen a 4.6-fold increased risk when patients presented with reduced LV systolic function. Likewise, patients were at a 2.89-fold increased risk when presenting with an

Table 4. Clinical characteristics of LVNC patients

(modified according to: Schultze-Berndt et al., 2021 (40))

	Index patients and family members			Pediatric and adult patients			
	Index patients n=113	**Affected family members n=36**	**All n=149**	**<18 years at diagnosis n=55 (40%)**	**>18 years at diagnosis n=82 (60%)**	**All n=137**	**P-Value**
Female	40 (35)	21 (58)	61 (41)	26 (47)	28 (34)	54 (39)	0.123
Age at diagnosis (years)	28.8 (9.0-46.8)	25.1 (13.9-36.4)	27.8 (9.2-44.7)	1.9 (0.2-10.7)	40.3 (29.0-54.1)	27.8 (9.2-44.7)	**<0.001**
<18 years at diagnosis	43 (39)	12 (43)	55 (40)				
Body surface area (m^2)	1.67 (1.08-1.90)	1.65 (1.51-1.87)	1.66 (1.21-1.90)	0.95 (0.33-1.43)	1.81 (1.63-1.96)	1.64 (1.15-1.89)	**<0.001**
Symptomatic	64 (62)	12 (43)	76 (58)	17 (34)	51 (69)	68 (55)	**<0.001**
Congenital heart defect	23 (20)	3 (8)	26 (17)	13 (24)	10 (12)	23 (17)	0.079
Ventricular septal defect	12 (11)	0 (0)	12 (8)	8 (15)	3 (4)	11 (8)	**0.027**
Patent foramen ovale	8 (7)	3 (8)	11 (7)	7 (13)	3 (4)	10 (7)	0.089
Ebstein anomaly	5 (4)	0 (0)	5 (3)	2 (4)	3 (4)	5 (4)	1.000
Patent ductus arteriosus	5 (4)	0 (0)	5 (3)	4 (7)	0 (0)	4 (3)	**0.024**
Other congenital heart defects	5 (4)	0 (0)	5 (3)	1 (2)	3 (4)	4 (3)	0.649
Echocardiography							
Reduced LV systolic function	54 (50)	11 (32)	65 (46)	17 (33)	44 (55)	61 (46)	**0.012**

	Index patients and family members			Pediatric and adult patients			
	Index patients n=113	**Affected family members n=36**	**All n=149**	**<18 years at diagnosis n=55 (40%)**	**>18 years at diagnosis n=82 (60%)**	**All n=137**	**P-Value**
LV-EF (%)	45.5 (32.0-60.0)	53.0 (40.0-65.0)	47.6 (33.0-62.0)	57.0 (44.0-67.0)	43.0 (33.0-55.0)	46.8 (33.0-64.0)	**0.001**
Increased LVEDD	48 (49)	7 (29)	55 (45)	21 (45)	34 (45)	55 (45)	0.944
LVEDD (mm) (patients >18 years only)	55.0 (50.6-65.0)	50.0 (48.0-62.0)	54.0 (49.0-65.0)	39.0 (30.0-48.0)	54.0 (49.0-65.0)	50.0 (42.0-60.0)	**<0.001**
LVEDD (Z-score) (patients <18 years only)	1.97 (0.40-4.41)	0.93 (0.74-1.57)	1.66 (0.40-4.39)	1.66 (0.40-4.39)			
Increased LVEDD and reduced LV systolic function	33 (34)	6 (17)	39 (26)	11 (24)	28 (38)	39 (33)	0.113
Subtypes							
LVNC	40 (47)	12 (52)	52 (48)	13 (33)	32 (54)	45 (46)	
Dilated LVNC	27 (31)	8 (35)	35 (32)	10 (25)	22 (37)	32 (32)	**<0.001**
Hypertrophic LVNC	19 (22)	3 (13)	22 (20)	17 (43)	5 (9)	22 (22)	
ECG							
ST-Deprivation	16 (14)	4 (11)	20 (13)	3 (5)	17 (21)	20 (15)	**0.013**
T-Inversion	19 (17)	3 (8)	22 (15)	5 (9)	17 (21)	22 (16)	0.069
Bundle branch block	20 (21)	2 (10)	22 (19)	2 (5)	19 (28)	21 (19)	**0.002**
Arrhythmias	19 (17)	8 (22)	27 (18)	5 (9)	20 (24)	25 (18)	**0.007**
Atrial fibrillation	1 (1)	1 (3)	2 (1)	0 (0)	2 (2)	2 (2)	0.516

	Index patients and family members			Pediatric and adult patients			
	Index patients n=113	**Affected family members n=36**	**All n=149**	**<18 years at diagnosis n=55 (40%)**	**>18 years at diagnosis n=82 (60%)**	**All n=137**	**P-Value**
Atrioventricular block II°/III°	1 (1)	0 (0)	1 (1)	1 (2)	0 (0)	1 (1)	1.000
Supraventricular tachycardia	6 (5)	2 (6)	8 (5)	2 (4)	6 (7)	8 (6)	0.475
Ventricular tachycardia	14 (12)	5 (14)	19 (13)	4 (7)	13 (16)	17 (12)	0.135
ICD	21 (19)	5 (14)	26 (17)	2 (4)	22 (27)	24 (18)	**<0.001**
Follow-up (years)	5.6 (1.7-11.2)	5.4 (1.9-13.1)	5.6 (1.8-11.4)	3.5 (1.5-7.4)	7.8 (1.8-13.7)	5.6 (1.8-11.4)	**0.016**
Complications							
MACE	25 (22)	2 (6)	27 (18)	12 (22)	15 (18)	27 (20)	0.611
HTx	12 (11)	2 (6)	14 (9)	9 (16)	5 (6)	14 (10)	0.052
Death	11 (10)	0 (0)	11 (7)	2 (4)	9 (11)	11 (8)	0.121

Values are given as n (%) or median (interquartile range).

HTx = Heart transplantation, ICD = Implantable cardioverter defibrillator, LVEDD = Left ventricular end-diastolic diameter, LV = Left ventricular, LVNC = Left ventricular noncompaction, LV-EF = Left ventricular ejection fraction, MACE = Major adverse cardiac events

increased LVEDD. Multivariate analysis identified reduced LV systolic function as the stronger risk factor for MACE than increased LVEDD (Table 5). With a reduced LV-EF by 1%, the risk for MACE increases by 6% (HR: 0.94; CI 95%: 0.92-0.97; p-Value <0.001).

Out of the patients with normal LV systolic function (n=49) at first presentation, 10,2% (n=5) showed a LV systolic dysfunction at follow-up and 8.2% (n=4) suffered death or HTx. When first presenting with reduced LV systolic function (n=47), 17.0% (n=8) regained normal function at follow-up and 38.3% (n=18) suffered death or underwent HTx. With regard to patients with normal LVEDD at first presentation (n=38), 13.2% (n=5) developed an increased LVEDD at follow-up and 10.5% (n=4) suffered death or underwent HTx. Patients who showed LVEDD elevation at first presentation regained in 31.3% (n=15) a normal LVEDD and 29.2% (n=14) suffered death or HTx.

The analysis of event-free survival is shown in Figure 4. The event-free survival time was significantly shortened by reduced LV systolic function and an increased LVEDD.

Table 5. Risk for MACE

(modified according to: Schultze-Berndt et al., 2021 (40))

	univariate		multivariate	
	HR (95% CI)	**P-Value**	**HR (95% CI)**	**P-Value**
Reduced LV systolic function	4.60 (1.56-13.55)	**0.006**	4.20 (1.11-15.89)	**0.035**
Increased LVEDD	2.89 (1.04-8.04)	**0.042**	1.62 (0.54-4.86)	0.393

LV = left ventricular; LVEDD = left ventricular end-diastolic diameter; MACE = Major adverse cardiac events

3.1.3 Genetic characteristics

134 variants in 87/113 (77%) index patients were identified through NGS. 95 (71%) were classified as VUS, 24 (18%) as likely pathogenic variants, and 15 (11%) as pathogenic variants. Out of 48 total variants in the pediatric cohort, 71% were classified as VUS, 19% as likely pathogenic, and 10% as pathogenic (Table 6).

We observed variants in 46 different genes. Most variants were in sarcomere genes (n=57; 42.5%). Aside from that, genes from the functional groups of cellular signalling (n=14; 10.4%), desmosome genes (n=13; 9.7%) and Z-disc (n=13; 9.7%) genes were mostly affected. Variants most frequently occurred in the sarcomere genes *MYH7*, *TTN*, and *MYBPC3*. Most affected non-sarcomere genes were *ACTN2*, *DSP*, and

PRDM16. The distribution of variants is shown in Figure 1. A list of all (likely) pathogenic variants is published in Schultze-Berndt et al. 2021 (40).

Table 6. Variant burden of index patients

(modified according to: Schultze-Berndt et al., 2021 (40))

	<18 years at diagnosis n=43	**>18 years at diagnosis n=66**	**All index patients n=113**
Total variants, n	48	82	134
Total VUS, n	34	58	95
Total likely pathogenic variants, n	9	15	24
Total pathogenic variants, n	5	9	15
Patients with no variant	11 (26)	14 (21)	26 (23)
Patients with VUS only	20 (47)	29 (44)	52 (46)
Patients with (likely) pathogenic variants only	5 (12)	13 (20)	18 (16)
Patients with VUS and (likely) pathogenic variants	7 (16)	10 (15)	17 (15)

Values are given as n (%). VUS = Variant of uncertain significance

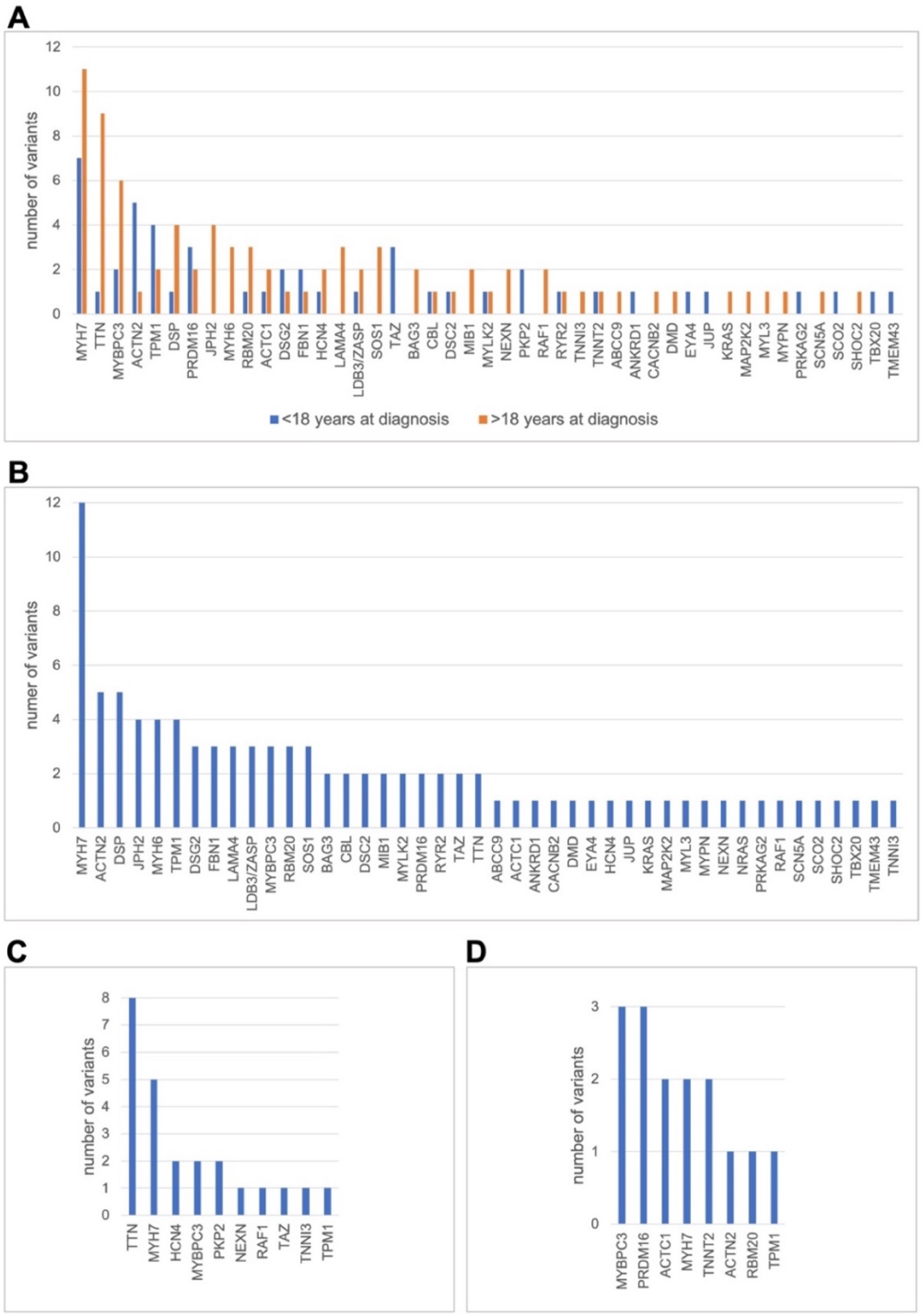

Figure 1. Number and distribution of genetic variants in index patients

(A): distribution of all variants between adult and pediatric index patients; (B): distribution of VUS; (C): distribution of likely pathogenic variants; (D): distribution of pathogenic variants. VUS = variant of uncertain significance. (Own illustration: Schultze-Berndt)

3.1.4 Impact of genetic factors on outcome

The presence or absence of variants did not increase or decrease the risk of developing a MACE, neither in the pediatric index patients nor the adult index patients. Likewise, the presence of sarcomere variants had no impact on the risk for MACE (Table 7).

The number of variants also did not make a difference in the risk for MACE. The event-free survival time was also not affected by the presence of one or multiple variants (Figure 2). Also, the groups 'no variant', 'VUS only', (likely) pathogenic variant only', and '(likely) pathogenic variant and VUS' did not show to be of impact in the Kaplan-Meier analysis (Figure 2).

Table 7. Risk of genetic factors for MACE.

(Own illustration: Schultze-Berndt)

	all		<18 years at diagnosis		>18 years at diagnosis	
	HR (95% CI)	P-Value	HR (95% CI)	P-Value	HR (95% CI)	P-Value
number of variants	0.76 (0.47-1.23)	0.262	1.28 (0.50-3.28)	0.610	0.59 (0.37-1.28)	0.181
no variant	1.57 (0.61-4.06)	0.349	0.84 (1.7-4.02)	0.822	2.27 (0.68-5.51)	0.181
VUS only	1.05 (0.45-2.43)	0.914	1.34 (0.36-5.00)	0.663	0.84 (0.27-2.56)	0.754
LP/P only	0.41 (0.10-1.78)	0.234	0.04 (0.00-508.50)	0.504	0.60 (0.13-2.73)	0.511
LP/P + VUS	1.09 (0.37-3.22)	0.877	1.55 (0.31-7.68)	0.593	0.86 (0.19-3.90)	0.846
MYH7 variant	0.19 (0.03-1.43)	0.108	0.04 (0.00-243.39)	0.468	0.29 (0.04-2.25)	0.236
TTN variant	0.38 (0.05-2.80)	0.339	0.05 (0.00-355164.64)	0.705	0.49 (0.06-3.75)	0.489
variant in sarcomere gene	0.73 (0.31-1.72)	0.473	2.76 (0.66-11.58)	0.165	0.39 (0.13-1.24)	0.110

LP = likely pathogenic; LV = left ventricular; LVEDD = left ventricular end-diastolic diameter; P = pathogenic; VUS = variant of uncertain significance

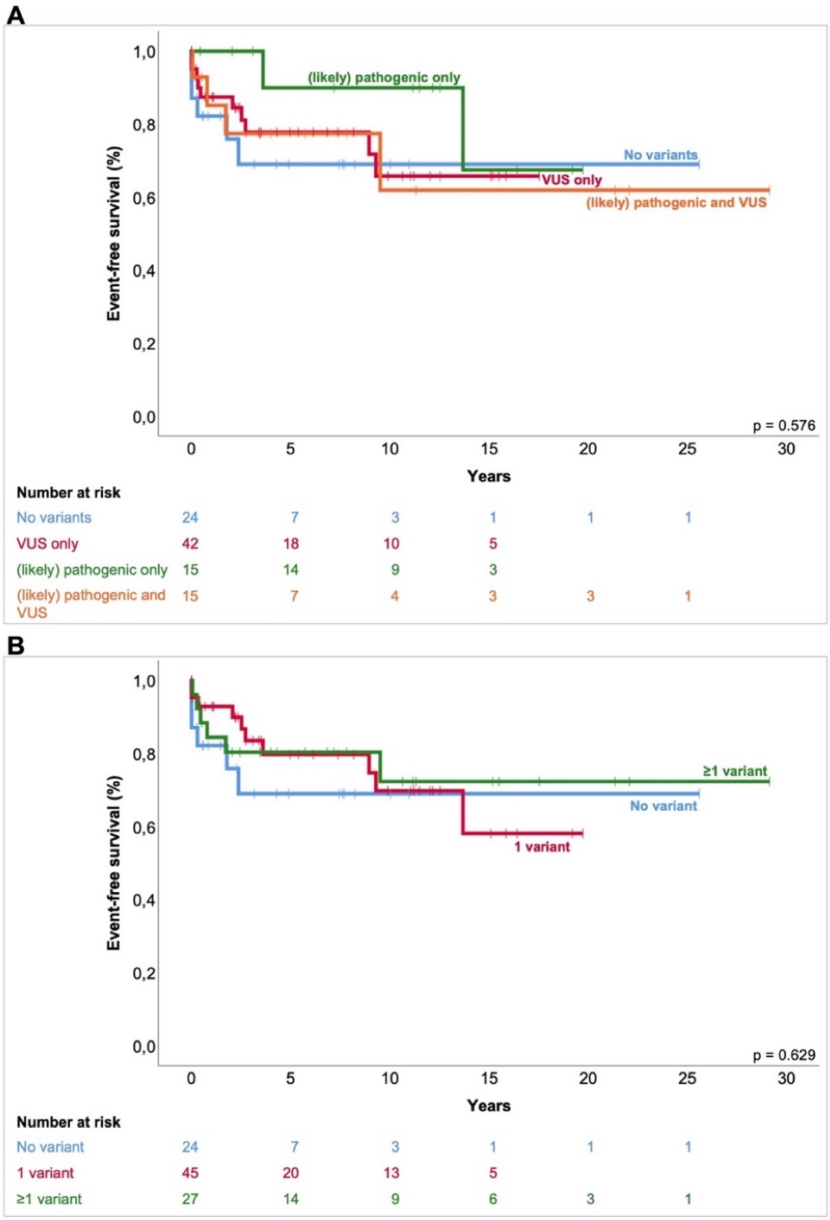

Figure 2. Event-free survival time (Kaplan-Meier analysis) for genotype.

(A): for the number of variants in 113 index patients; (B): for the groups 'no variant', 'VUS only', (likely) pathogenic variant only' and '(likely) pathogenic variant and VUS' in 113 index patients. VUS = variant of uncertain significance. (Own illustration: Schultze-Berndt)

3.1.5 Genotype-phenotype correlation

The presence or absence of variants did not show an impact on developing a reduced LV systolic function or an increased LVEDD. The number and pathogenicity of variants also did not influence these echocardiographic parameters. The phenotype was also not impacted by variants in specific genes (as shown for *MYH7* and *TTN*) or the presence of variants in sarcomere genes (Table 8).

Table 8. Risk of genetic factors on LV dysfunction and LVEDD elevation.

(Own illustration: Schultze-Berndt)

	Risk for reduced LV systolic function		Risk for increased LVEDD	
	OR (95% CI)	P-Value	OR (95% CI)	P-Value
number of variants	1.53 (0.90-2.59)	0.115	1.67 (0.95-2.92)	0.073
no variant	0.84 (0.35-2.02)	0.692	0.75 (0.29-1.92)	0.547
VUS only	0.71 (0.33-1.52)	0.381	1.08 (0.49-2.39)	0.855
LP/P only	1.34 (0.48-3.69)	0.577	0.61 (0.21-1.72)	0.346
LP/P + VUS	1.86 (0.62-5.53)	0.267	2.65 (0.76-9.29)	0.127
MYH7 variant	0.56 (0.19-1.67)	0.301	0.32 (0.09-1.10)	0.070
TTN variant	2.17 (0.51-9.15)	0.293	1.82 (0.41-8.08)	0.430
variant in sarcomere gene	1.50 (0.70-3.22)	0.294	1.27 (0.57-2.82)	0.556

LP = likely pathogenic; LV = left ventricular; LVEDD = left ventricular end-diastolic diameter; P = pathogenic; VUS = variant of uncertain significance

3.2 Subtypes

52/109 (48%) patients were classified into the group of isolated LVNC. 35/109 (32%) presented with dilated LVNC and 22/109 (20%) with hypertrophic LVNC. The distribution of sex between the different subtypes is even (percentage of female patients: isolated LVNC: 21/52 (40%); dilated LVNC 10/35 (29%); hypertrophic LVNC: 8/22 (36%); p-Value: 0.529). Also, no difference in the occurrence of symptoms was seen (isolated LVNC: 23/44 (52%); dilated LVNC: 22/32 (69%); hypertrophic LVNC: 9/19 (47%); p-Value: 0.232).

Patients with dilated LVNC suffered more often from arrhythmias (11/35 (31%); isolated LVNC: 9/52 (17%); hypertrophic LVNC: 4/22 (18%), p-Value: 0.264) and had more implantable cardioverter defibrillators (ICD) implanted (13/35 (37%); isolated LVNC: 6/52 (12%); hypertrophic LVNC: 3/22 (14%); p-Value: 0.010).

Hypertrophic LVNC was seen more frequently in the pediatric cohort (Table 4). Patients with hypertrophic LVNC had the highest rate of MACE, patients with isolated LVNC the lowest (4/52 (8%); dilated LVNC: 4/35 (11%), hypertrophic LVNC: 7/22 (32%); p-Value: 0.026). Being classified as hypertrophic LVNC put patients at a higher risk for MACE (OR: 4.61; CI 95%: 1.45-14.63; p-Value: 0.010). The event-free survival time between the subtypes is shown in Figure 3.

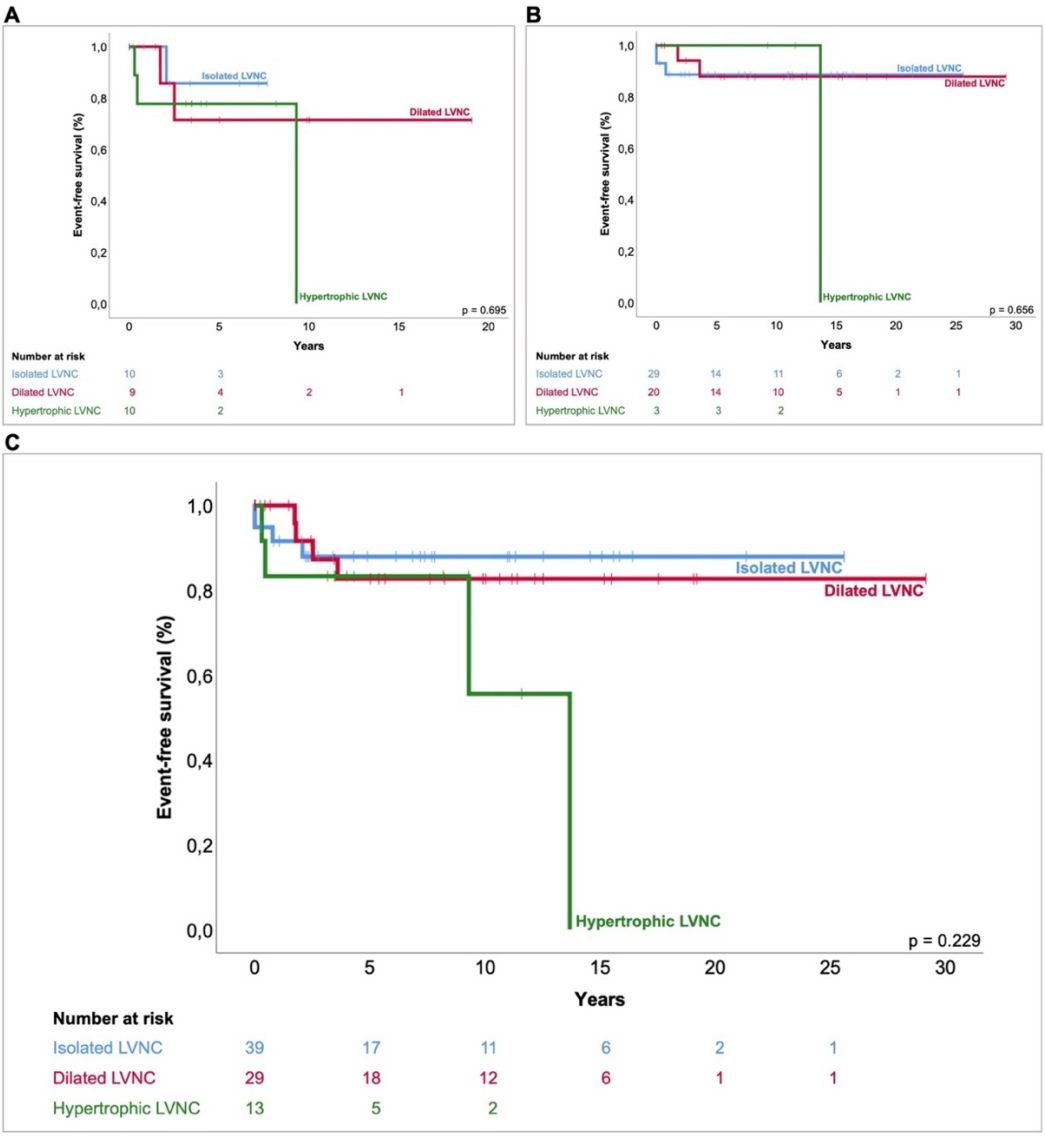

Figure 3. Event-free survival time (Kaplan-Meier analysis) for the LVNC subtypes.
(A): in patients <18 years at diagnosis; (B): in patients >18 years at diagnosis; (C): in all LVNC patients. LVNC = Left ventricular noncompaction. (Own illustration: Schultze-Berndt)

3.3 Pediatric cohort

In the pediatric cohort, the medium age was 1.9 (0.2-10.7) years at diagnosis. Compared to adult patients, pediatric patients were less often symptomatic. Also, we observed a lower rate of reduced LV systolic function and less ECG abnormalities (Table 4). The risk for MACE was not significantly different compared to the adult cohort (HR: 1.48; CI 95%: 0.63-3.50; p-Value: 0.372).

Over a median follow-up time of 3.5 (1.5-7.4) years, we observed 12 MACE in the pediatric cohort. The impact of LV-EF and LVEDD on the risk for MACE is shown in Table 9. In pediatric patients with a decreased BSA of 0.1m^2, the relative risk for MACE

increased by 8.4%. In adults, higher age increased the risk for MACE. Through multivariate analysis, LV-EF was identified as the stronger independent risk factor for MACE than LVEDD and BSA (Table 9).

Coherently, especially reduced LV systolic function had a significant effect on event-free survival time in the pediatric cohort. For adult patients, we could not see a significant impact of reduced LV systolic function or an increased LVEDD on event-free survival time (Figure 4).

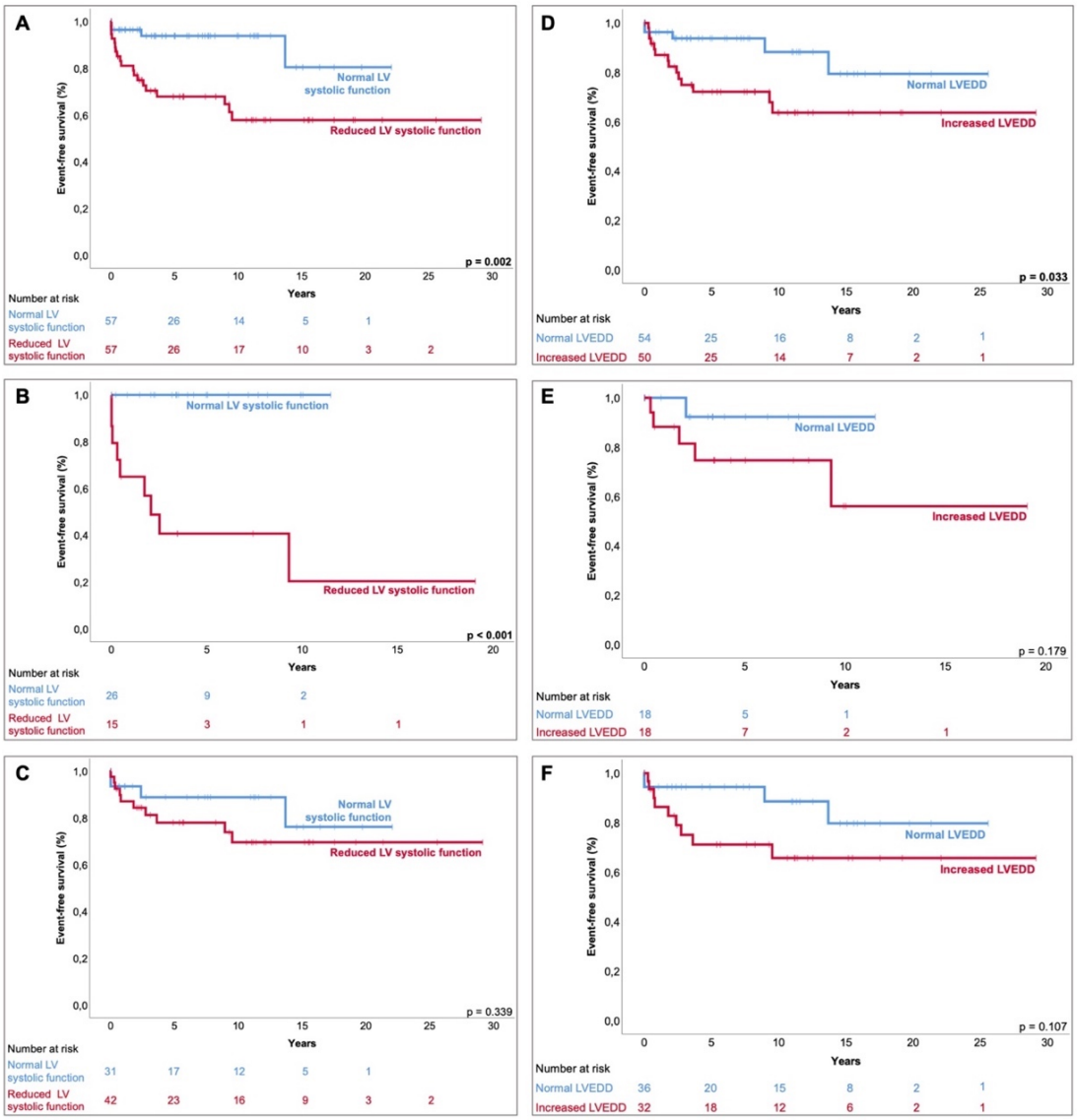

Figure 4. Event-free survival time (Kaplan-Meier analysis) for reduced LV systolic function and increased LVEDD.

Between the patient groups with normal LV systolic function and reduced LV systolic function in: (A) all patients; (B) pediatric patients; (C) adult patients. Between the patient groups of normal LVEDD and increased LVEDD in: (D) all patients; (E) pediatric patients; (F) adult patients. LV = left ventricular; LVEDD = left ventricular end-diastolic diameter. (Own illustration: Schultze-Berndt)

Table 9. Risk for MACE in pediatric patients

(modified according to: Schultze-Berndt et al., 2021 (40))

	univariate		multivariate	
	HR (95% CI)	**p-Value**	**HR (95% CI)**	**p-Value**
Body surface area (m^2)	0.16 (0.03-0.97)	**0.047**	0.03 (0.00-2.95)	0.130
LV-EF (%)	0.92 (0.87-0.96)	**0.001**	0.92 (0.86-0.99)	**0.032**
LVEDD (Z-score)	1.50 (1.39-1.98)	**0.004**	1.37 (0.90-2.09)	0.148

LVEDD = Left ventricular end-diastolic diameter, LV-EF = Left ventricular ejection fraction, MACE = Major adverse cardiac events

3.4 Family members

We included 202 family members from 54 families in our analysis. 36 family members had a diagnosis of LVNC, and 9 had a hypertrabeculated myocardium. 12/28 (43%) affected family members were under 18 years at diagnosis.

We performed Sanger sequencing for 85 different variants in 146 family members. Through sanger sequencing, we found 78 variant carriers among the family members. Out of the family members diagnosed with LVNC, 30/36 (83%) were variant carriers, and 6/36 (17%) did not carry variants or were not tested. Among the family members with a definite exclusion of LVNC, 37/60 (62%) carried at least one of their index patients variant.

An example for the the screening of family members is shown for family 9 in Figure 5. The variant *TTN* c.63601C>T, p.Arg21201* was identified in the index patient 9-I:1 through NGS. The index´es sister (9-I:2) and nephew (9-II:1) were also diagnosed with LVNC. Through sanger sequencing, we identified both of them as variant carriers.

Another example is family 2. In the case of family 2, the variant *MYBPC3* c.1484G>A, p.Arg495Gln was identified in the index patient 2-I:1 and through family screening in the affected family member 2-II:1. Individual 2-III:1 carrying the variant and not showing the phenotype indicates incomplete penetrance of the variant (Figure 5).

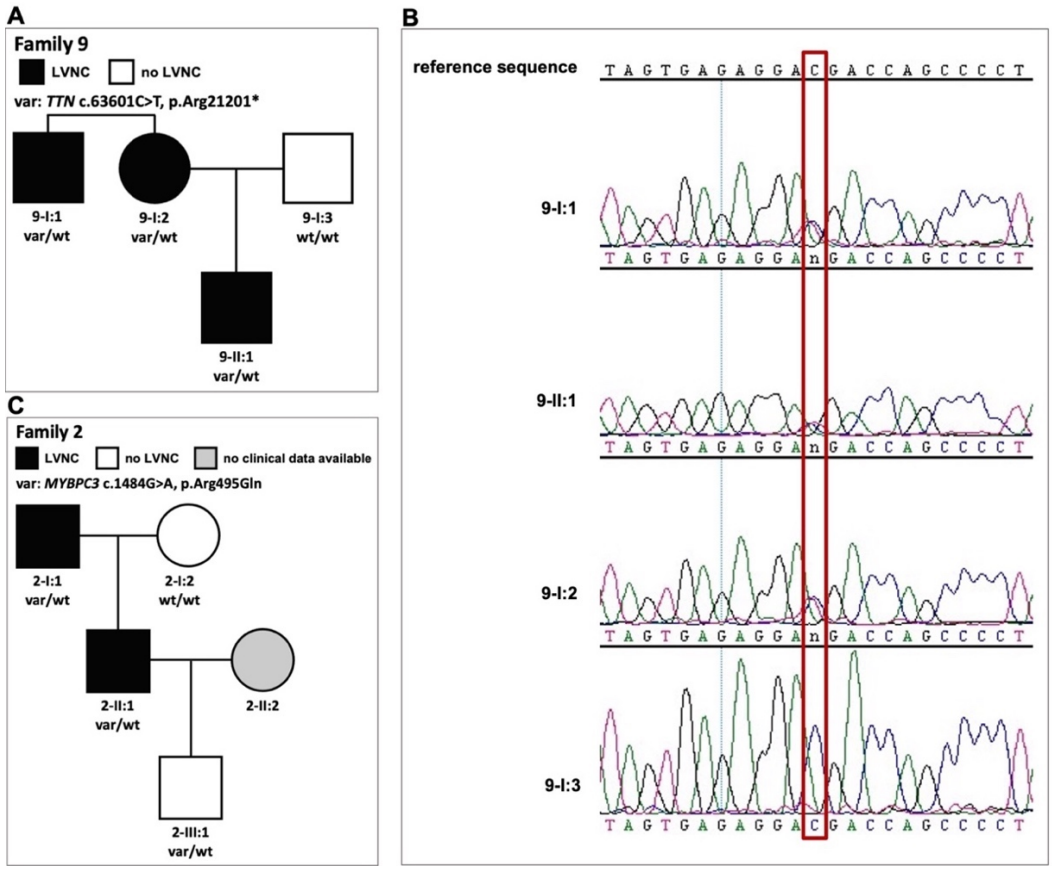

Figure 5. Example for result from genetic family screening

(A) Pedigree of family 9; (B) Result of sanger sequencing for family 9; (C) Pedigree of family 2. LVNC = Left ventricular noncompaction; var = variant; wt = wildtype. (Own illustration: Schultze-Berndt)

4 Discussion

In our cohort comprising 149 patients with LVNC, we searched for clinical and genetic risk factors and specific LVNC subtypes that lead to an adverse outcome. We identified the phenotype of reduced LV systolic function as the main risk factor for the appearance of MACE. This high-risk phenotype was found likewise in children and adults (Figure 6). The genotype did not predict the clinical outcome in pediatric or adult patients.

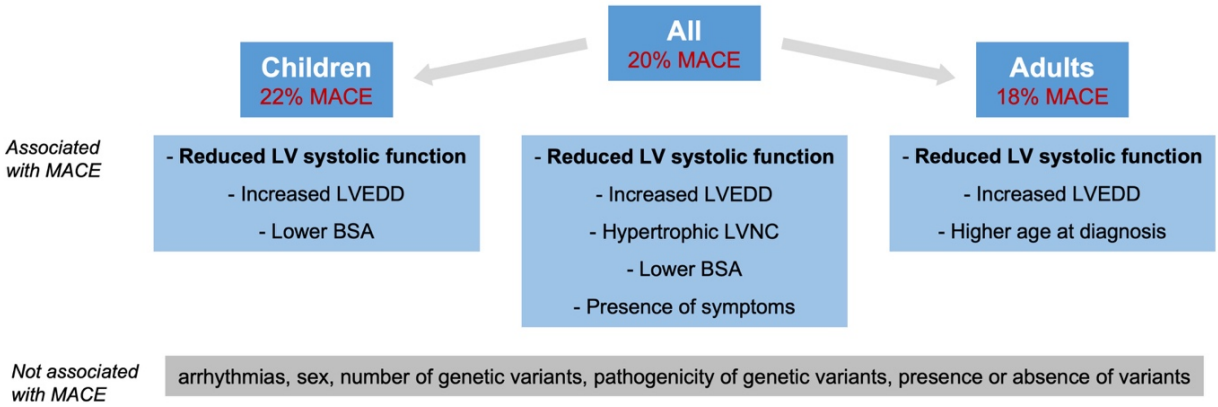

Figure 6. Main risk factors for MACE in pediatric and adult LVNC.

Reduced LV systolic function is the main risk factor for MACE in the pediatric, adult, and overall cohort of patients with LVNC. Genetic factors were not predictive for the risk of developing a MACE. BSA = Body surface area; LVEDD = Left ventricular end-diastolic diameter; LVNC = Left ventricular noncompaction; MACE = Major adverse cardiac event. (Own illustration: Schultze-Berndt)

4.1 Clinical risk factors for adverse outcome

During the median follow-up of 5.6 years, our cohort's mortality rate was 7%, 4% in pediatric LVNC, and 11% in adult LVNC. This is lower than reports of 12-20 % mortality rate for children in other studies (18, 24, 41, 42), in adults, 17% are reported (23). The lower mortality rate might be due to our retrospective approach with a very variable follow-up time; in some patients no follow-up time was available.

In a systematic review of 2501 adult LVNC patients, Aung et al. identified LV-EF as the main risk factor for adverse outcome. They report the extent of trabeculation having no impact on the prognosis (16). Other clinical risk factors associated with adverse outcome in adult patients were positive family screening, older age at diagnosis, male sex, and specific ECG abnormalities (14).

In children, a higher risk for MACE has been reported for age under one year at diagnosis, younger age in general, lower BSA, ventricular dysfunction, increased end-diastolic dimension, compaction of the LV posterior wall, ventricular arrhythmias, and various ECG abnormalities (5, 15, 24, 43). We could not confirm all of the risk factors mentioned above in our pediatric cohort, but we could see an effect for lower BSA and therefore smaller and younger children, elevated LVEDD, and reduced systolic function. Also, the absence of symptoms reduced the risk for MACE significantly.

The risk for MACE and event-free survival time was not related to sex in our cohort. More cardiac heart arrests and ischemic strokes have been described in women, but the data on this matter is generally inconsistent (5, 14). At this point, sex cannot be used for proper risk stratification.

The percentage of 50% of patients with reduced LV systolic function is a little less compared to other cohorts. Therefore, it might partly explain the lower mortality in our cohort. Chin et al., who first described eight cases with isolated noncompaction of the left ventricular myocardium in 1990, reported reduced left ventricular function in 63%, Van Waning et al. reported 58% in adult patients, Brescia et al. reported 62% in pediatric LVNC (1, 5, 24).

Our results underline the critical role of LV systolic function in outcome prediction in LVNC. Likewise, many other current publications have seen this correlation in adults (14, 16, 23) and children (15, 24, 44). In line with this, it appears that patients with a normal LV systolic function have a very good prognosis, especially when the other echocardiographic parameters such as LV wall-thickness and end-diastolic diameter are normal. Patients with normal LV-EF and an isolated apical noncompaction can be expected to have a comparable survival time to the average population without LVNC (23). A small chance for sudden death is described in pediatric patients with a normal LV diameter and normal LV function (24). In our cohort, patients with normal LV systolic function suffered distinctly less often from HTx or death (8.2% versus 38.3%). However, it must still be noted that adverse events occur in patients with normal LV systolic function and therefore need further investigation for more precise judgment of the disease (24).

4.2 Imaging modalities

Generally, echocardiography is the most widely available and therefore the most used tool for the diagnosis of LVNC. Most widely used for diagnosis are the criteria according

to Jenni (8). Most commonly applied for CMR imaging are the criteria by Petersen et al. (22). The use of different diagnostic criteria may reduce the comparability of different studies.

For echocardiography, poor interobserver reproducibility of results has been described, and to establish the diagnosis by correct, reproducible measurements is aggravated (45). Besides echocardiography, CMR imaging is essential in diagnosing LVNC and the morphological assessment of the disease. The evolving imaging technologies with much-improved image resolution has led to an increasing number of diagnosis, sometimes referred to as overdiagnosis (46). Another reason for the reported increasing number of patients diagnosed with LVNC in recent years might be the raising awareness of the disease (46).

The number of trabeculations does not influence the likelihood of MACE to occur (45). While not included in our study, existing studies show that the extent of trabeculation does not influence the outcome of patients nor the development of a reduced systolic function (47, 48). Therefore, not the absolute ratio of noncompacted to compacted myocardium should be the primary focus for risk stratification when CMR imaging is used. More data emphasize the importance of late gadolinium enhancement (LGE) in LVNC. A prospective, multicenter study identified the presence and extent of LGE as a risk factor for LV systolic dysfunction and abnormal clinical findings, such as symptoms of heart failure, ECG- and 24h-ECG-abnormalities in adults (49). Therefore, it has to be assumed that LGE is also associated with a worse outcome, which is confirmed by other studies (48, 50). A meta-analysis by Grigoratos et al. did even come to the conclusion that no hard cardiac events occur in the absence of LGE and LV systolic function (50). This underlines the importance of CMR imaging for risk stratification.

LGE can be found in 10-42% of pediatric patients (50, 51). Nevertheless, it can possibly only be used in older children and adolescents, as LGE seems to not occur in neonates and young children (52). For these patients, other diagnostic tools are needed for risk stratification.

4.3 Pediatric LVNC versus Adult LVNC

Comparing our pediatric to the adult cohort, they were less often symptomatic and had lower rates of reduced LV systolic function. Incoherent to other studies, we did not see a worse outcome in children, which might be explained by our older pediatric cohort

(median age in children: 1.9 years versus 0 years) and exclusion of children with (neuromuscular) syndromes and chromosomal abnormalities (43). The age alone did not elevate the risk for MACE, but lower BSA did, and therefore overall younger children with lower body weight. In pediatric and adult patients, we did not see a statistically significant impact of the presence of symptoms, like in the overall cohort. It can be expected to be caused by the smaller sizes of these subcohorts and, therefore, lack of statistical power.

In pediatric cohorts, a more important role of genetic factors is described than in adults, and overall more genetic cases (5, 26). In our cohort, we were not able to confirm these results.

4.4 Subtypes

We sorted the patients into subtypes based on the classification used by Van Waning et al. (27). A higher incidence of MACE is reported for the dilated subtype in mixed and pediatric-only cohorts (18, 27). This might be explained by LV dilation resulting from severe heart failure, as in other diseases of the heart. The lowest event-free survival time in pediatric patients was seen for the dilated and mixed phenotype (24). On the contrary, in our cohort, patients with hypertrophic LVNC suffered from the most events classified as MACE. Patients with hypertrophic LVNC were also a lot younger than the patients presenting with the other phenotypes. In the pediatric cohort, we could not see a significant impact of the hypertrophic phenotype on the occurrence of MACE like in the whole cohort. This might be due to the smaller cohort size.

For the time being, the LVNC subtypes used by us cannot help with precise disease management. Furthermore, LV function might need to be added for subtype classification, as the data is clear on its relevance for risk stratification.

Patients with normal cardiac dimensions have lower mortality than patients with abnormal cardiac dimensions. This was also seen in other studies and is coherent with our data (18, 24). It must be suspected that in the group with isolated LVNC there are some patients with a noncompacted myocardium without ever developing cardiomyopathy (53). To identify those patients, it is suggested to apply functional parameters additionally to the morphologic diagnostic criteria.

4.5 Genes

For approximately half of the patients with LVNC, a genetic cause can be suspected (5, 54). In our cohort, we found variants in 77% of index patients. Pathogenic variants can be found in 38% (25, 29). In some studies, the number of variants correlated with markers of disease severity, such as reduced LV systolic function and the extent of noncompaction (54). For pathogenic variants in children, a higher risk for death, HTx, and ICD implantation is described (29). The presence of multiple variants is described as a risk factor for MACE (44).

Around 80 different genes involved have been reported (43). Our study's most commonly affected genes (*MYH7*, *MYBPC3*, and *TTN*) are coherent with other studies (5, 25). Variants in *TTN* occurred in our cohort almost exclusively in adult patients. This has been shown for DCM before and can possibly be explained by a milder phenotype caused by variants in *TTN* which leads to a higher age of onset (55). Patients with a variant in *MYH7* and *ACTC1* have a lower risk for MACE than those with variants in other sarcomere genes (43). Meanwhile, a poorer outcome is associated with variants in *MYBPC3*, *TTN*, *LMNA*, *RBM20*, *TAZ*, and for truncating variants in *TTN* (5, 25, 29, 43, 56, 57).

Many studies show that variants in sarcomere genes are the most common variants in LVNC. In some studies, there was an association between sarcomere genes and an increased LGE and therefore a possibly worse outcome seen (54). Also, more frequent LV systolic dysfunction for patients with a variant in a sarcomere gene was described (43). On the other hand, there are also publications with a higher rate of death and heart transplantation in patients with a non-sarcomere variant compared to patients with a sarcomere variant (56). The same was described by Wang et al. for a cohort of 102 children, who have seen a better prognosis for children with a sarcomere variant (29).

Multiple genotype-phenotype correlations have been described in the past. Some studies have seen more LV systolic dysfunction in patients with a pathogenic variant (5, 29). It has been observed that patients with variants in ion channel genes are more likely to have arrhythmias (44). Patients with a variant in *MYH7* and *TAZ*, which are frequently affected in LVNC, showed different phenotypes (29). The same has been shown for sarcomere genes, which are, despite their important role in LVNC, not helpful in predicting a clinical phenotype (58).

We could not confirm the abovementioned associations between genotype and phenotype or genotype and outcome. It could be due to our smaller cohort, especially when only looking at the genetic cases. However, it might still hint that described associations are small and still not enough to predict the course of disease in individual cases clearly. Further studies, including more detailed phenotyping in larger cohorts, are needed to draw better conclusions for predicting the course of disease and outcome in individual patients. Additionally, more precise family counseling would be possible. For now, only vague assumptions can be made based on the results of genetic testing.

It is currently recommended to run genetic testing using gene panels developed for DCM or HCM, according to the specific LVNC phenotype the patient is showing (28). Meanwhile, with many studies underlying the benign nature of a noncompacted phenotype in otherwise asymptomatic individuals without any other cardiac findings, genetic testing is not recommended in those individuals (59, 60).

4.6 Role of family screening

About half of the patients diagnosed with LVNC by family screening are reported to be asymptomatic (27). In our retrospective study, no systematic family screening was performed, but we also saw fewer symptomatic affected family members (43%) than symptomatic index patients (62%). This may lead to falsely low results of family screening, as some family members may not see the need for diagnostics in the absence of symptoms.

The role of non-penetrance of variants must also be discussed. Van Waning et al. describe non-penetrance of variants in 37% of family members carrying a variant (27), while we observed non-penetrance in 47% of variant carriers among the family members. However, it must be noted that some of these family members had only one of their index patients' multiple variants. In these cases, one of the variants might function as a genetic modifier, altering the development of the disease and not cause LVNC by itself.

4.7 Limitations

Clinical data were collected from different physicians' records and might lack comparability because of other assessment criteria for LVNC. Due to our retrospective approach, some clinical findings, comorbidities, and other affected family members

may have been found later, leading to lower numbers in our analysis. For many family members, no clinical data were available.

We cannot rule out a referral bias, as the included patients were mainly treated in tertiary referral centers and might have been affected more severely. Also, patients were diagnosed over a period of 30 years, in which the improvement of imaging modalities might have led to data with poorer comparability. Because of our limited cohort size and partly missing data, small subgroups led to a limitation of statistical power. Comparing pediatric and adult patients is complex and resulted in the need for the transformation of numerical data into dichotomous variables.

5 Conclusions

Our retrospective analysis aimed to identify predicting factors for outcome, phenotype, and overall course of disease in children and adults with LVNC. We identify reduced LV systolic function as the main independent risk factor for MACE in pediatric and adult LVNC. In the pediatric cohort, univariate analysis also recognizes a lower BSA and an increased LVEDD as factors elevating the risk for MACE. The genetic findings did not correlate with the outcome or specific phenotypes of the disease. When family screening for identifying family members at risk for developing LVNC is performed, non-penetrance of variants plays an important role.

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Eidesstattliche Versicherung

Ich, Alina Katharina Schultze-Berndt, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: Patients with Left Ventricular Noncompaction Cardiomyopathy - Comparison between Pediatric and Adult Phenotypes (Patienten mit linksventrikulärer Noncompaction Kardiomyopathie - Vergleich des Phänotyps zwischen Erwachsenen und Kindern) selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.

Datum Unterschrift

Anteilserklärung an den erfolgten Publikationen

Alina Katharina Schultze-Berndt hatte folgenden Anteil an der folgenden Publikation:

Publikation:
Schultze-Berndt, A., Kühnisch, J., Herbst, C., Seidel, F., Al-Wakeel-Marquard, N., Dartsch, J., Theisen, S., Knirsch, W., Jenni, R., Greutmann, M., Oechslin, E., Berger, F., & Klaassen, S., Reduced Systolic Function and Not Genetic Variants Determine Outcome in Pediatric and Adult Left Ventricular Noncompaction Cardiomyopathy. Frontiers in pediatrics, 2021

Beitrag im Einzelnen:
Die Planung der Studie habe ich gemeinsam mit meiner Erstbetreuerin, Frau Prof. Dr. Klaassen durchgeführt. Ich habe die erste Version des Manuskriptes der Publikation verfasst, die daraufhin gemeinsam mit den Co-Autorinnen und Co-Autoren überarbeitet wurde. Die finale Version des Manuskripts und die Revisionen wurden von meiner Erstbetreuerin erstellt und eingereicht. Die klinischen Daten aller eingeschlossenen Patientinnen und Patienten wurden von mir zusammengestellt. Die genetische Analyse mittels Next Generation Sequencing und dessen Auswertung wurde nicht von mir durchgeführt, ebenso die Bewertung der Varianten. Die genetische Analyse mittels Sanger Sequenzierung wurde teilweise von mir durchgeführt.
Die gesamte statistische Auswertung wurde von mir durchgeführt. Daraus sind die Tabellen 1, 2, 3 und 4 und die Supplemental Tables 1, 2, 3 und 4 entstanden. Ebenso basieren die Abbildungen 1, 2, 3 und 4 und die Supplemental Figure 1 auf der von mir durchgeführten Datenanalyse und statistischen Auswertung und wurden von mir erstellt.

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Unterschrift, Datum und Stempel der erstbetreuenden Hochschullehrerin

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Unterschrift der Doktorandin

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Journal Data Filtered By: **Selected JCR Year: 2019** Selected Editions: SCIE,SSCI
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1	JAMA Pediatrics	9,795	13.946	0.045070
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5	PEDIATRICS	79,434	5.359	0.096780
6	PEDIATRIC ALLERGY AND IMMUNOLOGY	4,456	4.699	0.005920
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8	EUROPEAN CHILD & ADOLESCENT PSYCHIATRY	5,422	3.941	0.009450
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13	Pediatric Obesity	2,306	3.429	0.005900
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15	PEDIATRIC DIABETES	4,017	3.052	0.009180
16	ARCHIVES OF DISEASE IN CHILDHOOD	16,291	3.041	0.013580
17	JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION	12,405	2.937	0.016740
18	PAEDIATRIC AND PERINATAL EPIDEMIOLOGY	3,398	2.917	0.004690
19	PEDIATRIC NEUROLOGY	5,578	2.890	0.008460
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24	PEDIATRIC RESEARCH	13,816	2.747	0.013390
25	Neonatology	2,856	2.742	0.005390
26	Paediatric Respiratory Reviews	1,714	2.716	0.002700
27	BIRTH-ISSUES IN PERINATAL CARE	2,440	2.705	0.002500
28	PEDIATRIC NEPHROLOGY	9,325	2.676	0.009770
29	Frontiers in Pediatrics	2,922	2.634	0.009360
30	Pediatric Rheumatology	1,385	2.595	0.003840
31	Childhood Obesity	1,385	2.548	0.003930
32	International Breastfeeding Journal	1,079	2.545	0.001760
33	PEDIATRIC PULMONOLOGY	6,764	2.534	0.009060
34	PEDIATRIC DRUGS	1,258	2.519	0.002150

Druckexemplar der Publikation

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ORIGINAL RESEARCH
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Reduced Systolic Function and Not Genetic Variants Determine Outcome in Pediatric and Adult Left Ventricular Noncompaction Cardiomyopathy

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Background: Left ventricular noncompaction cardiomyopathy (LVNC CMP) is a genetic cardiomyopathy. Genotype-phenotype correlation and clinical outcome of genetic variants in pediatric and adult LVNC CMP patients are still unclear.

Methods: The retrospective multicenter study was conducted in unrelated index patients with LVNC CMP, diagnosed between the years 1987 and 2017, and all available family members. All index patients underwent next-generation sequencing for genetic variants in 174 target genes using the Illumina TruSight Cardio Sequencing Panel. Major adverse cardiac events (MACE) included mechanical circulatory support, heart transplantation, survivor of cardiac death, and/or all-cause death as combined endpoint.

Results: Study population included 149 LVNC CMP patients with a median age of 27.8 (9.2–44.8) years at diagnosis; 58% of them were symptomatic, 18% suffered from non-sustained and sustained arrhythmias, and 17% had an implantable cardioverter defibrillator (ICD) implanted. 55/137 patients (40%) were $\leq$18 years at diagnosis.
A total of 134 variants were identified in 87/113 (77%) index patients. 93 variants were classified as variant of unknown significance (VUS), 24 as likely pathogenic and 15 as pathogenic. The genetic yield of (likely) pathogenic variants was 35/113 (31%) index patients. Variants occurred most frequently in *MYH7* (n=19), *TTN* ($n = 10$) and *MYBPC3* ($n = 8$). Altogether, sarcomere gene variants constituted 42.5% ($n = 57$) of all variants. The presence or absence of (likely) pathogenic variants or variants in specific genes did not allow risk stratification for MACE.
Reduced left ventricular (LV) systolic function and increased left ventricular end-diastolic diameter (LVEDD) were risk factors for event-free survival in the Kaplan-Meier analysis.

Through multivariate analysis we identified reduced LV systolic function as the main risk factor for MACE. Patients with reduced LV systolic function were at a 4.6-fold higher risk for MACE.

Conclusions: Genetic variants did not predict the risk of developing a MACE, neither in the pediatric nor in the adult cohort. Multivariate analysis emphasized reduced LV systolic function as the main independent factor that is elevating the risk for MACE. Genetic screening is useful for cascade screening to identify family members at risk for developing LVNC CMP.

Keywords: cardiomyopathy, pediatric and congenital heart disease, genetics, noncompaction, pediatrics - children

INTRODUCTION

Left ventricular noncompaction cardiomyopathy (LVNC CMP) is a rare genetic cardiomyopathy. LVNC is characterized by prominent trabeculations and deep intertrabecular recesses communicating with the left ventricular cavity; a two-layered myocardium with an at least twice as thick non-compacted than the thinned compacted layer are mandatory phenotypic characteristics (1). LVNC CMP is diagnosed in all age groups (2–4). In children, LVNC CMP is reported to make up around 5–10 % of cardiomyopathies (5, 6). For adults, an incidence of 0.05% was described (7) and the five-year survival was reported to be around 86% (8). LVNC CMP is a very heterogenous clinical disease ranging from asymptomatic to severely affected patients with the need for heart transplantation (Htx) or the risk for sudden cardiac death. Typical symptoms and complications are congestive heart failure, arterial thrombembolism, arrhythmias, and sudden cardiac death (9–11). The diagnosis is mostly made by routine transthoracic 2D Doppler echocardiography and cardiac magnetic resonance (CMR) imaging. Currently, it is difficult to predict the clinical course of the disease. Due to the clinical heterogeneity, it is important to identify high risk patients at an early stage.

Approximately in 50% of patients LVNC has a genetic cause (4). It has been known for a while that sarcomere genes are affected most frequently with around 63% of relevant variants identified (12, 13). A large part of the genetic variants found were also associated with other cardiomyopathies, such as dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) (14, 15). A recent study reported LVNC specific truncating variants in *MYH7*, *ACTN2* and *PRDM16* (16). Current guidelines recommend genetic testing, although the specific therapeutic implications of the results remain mostly unknown (17).

Van Waning et al. divided the LVNC phenotype into 3 groups, differentiating isolated LVNC CMP from LVNC with DCM and LVNC with HCM (18). It remains unclear whether patients, who phenotypically belong to one of these groups can expect a similar course of disease as patients with DCM or HCM without LVNC. So far, the general incidence of adverse events in adults with LVNC CMP was described being similar to DCM without LVNC, with a slightly higher heart failure admission rate (19). Furthermore, the question remains whether and to what extent the different subtypes of LVNC correlate with genetic equivalents.

In this study, we examined genetics, clinical phenotype and outcome of 113 pediatric and adult index patients with LVNC CMP and their family members. We analyzed retrospective data to compare risk factors for adult and pediatric patients and different subtypes of LVNC CMP to further classify patients for more individual risk stratification and individual therapeutic regimes.

Abbreviations: BSA, Body surface area; CMR, Cardiac magnetic resonance; DCM, Dilated cardiomyopathy; HCM, Hypertrophic cardiomyopathy; ICD, Implantable cardioverter defibrillator; LVEDD, Left ventricular end-diastolic diameter; LV, Left ventricular; LVNC, Left ventricular noncompaction cardiomyopathy; LV-EF, Left ventricular ejection fraction; MACE, Major adverse cardiac events; NGS, Next-generation sequencing; VUS, Variant of uncertain significance.

METHODS

Study Population

The retrospective study consisted of unrelated index patients with LVNC CMP diagnosed between the years 1987 and 2017. Additionally, we included all available affected and unaffected family members. Clinical data was collected through medical records from Charité-Universitätsmedizin Berlin and German Heart Center Berlin, Germany and University Hospital Zurich and University Children's Hospital Zurich, Switzerland. The study was approved by the institutional ethics committees in accordance with the Declaration of Helsinki. All participants and legal guardians of participants under 18 years gave written informed consent.

Genetic Testing

All index patients underwent next-generation sequencing (NGS) for genetic variants in 174 target genes using the Illumina TruSight Cardio Sequencing Panel. Eighty-nine cardiomyopathy genes were bioinformatically filtered as previously published (20) with a minor allele frequency (MAF) of <0.0001 (gnomAD reference database, *https://gnomad.broadinstitute.org/*). Variants were classified according to the guidelines of the American College of Medical Genetics and Genomics (21). Unaffected and affected family members underwent Sanger Sequencing for the variants identified in the respective index patients. These variants included variants classified as (likely) pathogenic and variants

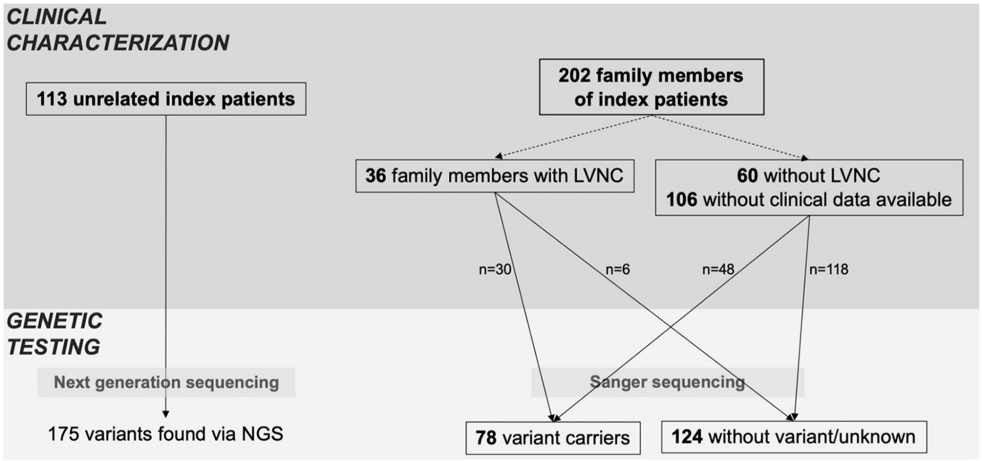

FIGURE 1 | Index patients and family screening. Overview of clinical characterization and genetic testing of patients, including all available family members. Family members were classified as variant carriers, when they carried at least one of the variants of the respective index patient.

of uncertain significance (VUS). The 89 genes which were bioinformatically evaluated were sorted into functional groups as previously published by Kühnisch et al. (20). The index patients were classified into four groups according to the presence of genetic variants: a) patients with no variants; b) patients with only VUS variants; c) patients with only (likely) pathogenic variants; and d) patients with VUS and (likely) pathogenic variants.

Diagnostic Criteria

LVNC was diagnosed by echocardiography according to the gold standard by Jenni et al. (1). The patients were classified into three phenotypic subtypes: Isolated LVNC CMP, dilated LVNC CMP and hypertrophic LVNC CMP (18). For adult patients, dilated LVNC CMP was diagnosed in patients with an increased left ventricular end-diastolic diameter (LVEDD) $\geq$54 mm in females and $\geq$60 mm in males (22). Hypertrophic LVNC CMP was defined by a left ventricular (LV) wall-thickness $\geq$13 mm (23). For pediatric patients, we used LVEDD and LV wall-thickness >2 standard deviations different from a normal population (24). When both, increased LVEDD and increased LV wall-thickness were found at the same time, we classified the patient as hypertrophic LVNC CMP. Patients with neither increased LVEDD nor increased LV wall thickness were categorized as isolated LVNC CMP. When the values for LVEDD or wall thickness were not available, the patients were excluded from the subtype analysis. Reduced LV systolic function was defined as LV ejection fraction (LV-EF) <45% or fractional shortening <19% in males and <21% in females (22).

Follow-up. Follow-up for occurrence of major adverse events (MACE) started with the date of diagnosis including mechanical circulatory support (MCS), HTx, survival of sudden cardiac death, and/or all-cause death as a combined endpoint. Event-free survival was defined as the time to MACE. When dyspnoe, syncope, shock, or palpitations were recorded patients were classified as symptomatic. Arrhythmias included atrioventricular block II°/III°, non-sustained and sustained supraventricular tachycardia, non-sustained and sustained ventricular tachycardia, and atrial fibrillation recorded by 12-lead ECG or Holter-ECG. Body surface area (BSA) was calculated using the Mosteller method (25).

Statistical Analysis

Statistical analysis was performed using SPSS v.26 (IBM Corporation). For categorical data we used the Pearson x^2 test. For tables with an expected cell frequency <5, the Fisher exact test was used. Continuous data was compared with the Mann-Whitney U test for 2 independent groups and the Kruskal-Wallis test for >2 independent groups. Odd ratios were calculated using binary logistic regression. For Hazard ratios (HR) we performed Cox regression analysis. Kaplan-Meier curves were used for event-free survival analysis with the time of diagnosis as time point zero. The survival times of different groups were compared with the log-rank test. In the survival analysis, patients were considered at risk until the time of last follow-up, at which they were censored.

RESULTS

Clinical Characteristics of LVNC Patients

As shown in **Figure 1**, the cohort consisted of 113 unrelated LVNC CMP patients and 202 family members from individual 54 families. Clinical data were available for 96 family members, of which 36 (37.5%) had a diagnosis of CMP and 9 (9.4%) had

TABLE 1 | Clinical characteristics of LVNC patients.

	All *n* = 149
Female	61 (41)
Age at diagnosis (yrs)	27.8 (9.2–44.8)
<18 years at diagnosis	55 (40)
Body surface area (m^2)	1.66 (1.21–1.90)
Symptomatic	76 (58)
Congenital heart defect	26 (17)
Ventricular septal defect	12 (8)
Patent foramen ovale	11 (7)
Ebstein anomaly	5 (3)
Patent ductus arteriosus	5 (3)
Other congenital heart defects	5 (3)
Echocardiography	
Reduced LV systolic function	65 (46)
LV-EF (%)	47.6 (33.0–62.5)
Increased LVEDD	55 (37)
LVEDD (mm)(patients >18 yrs only)	54.0 (49.0–65.0)
LVEDD (Z-score)(patients <18 yrs only)	1.66 (0.40–4.39)
Increased LVEDD and reduced LVsystolic function	39 (26)
Subtypes	
Isolated LVNC	52 (48)
Dilated LVNC	35 (32)
Hypertrophic LVNC	22 (20)
ECG	
ST-Depression	20 (13)
T-Inversion	22 (15)
Bundle branch block	22 (15)
Arrhythmias	27 (18)
Atrial fibrillation	2 (1)
Atrioventricular block II°/III°	1 (1)
Supraventricular tachycardia	8 (5)
Ventricular tachycardia	19 (13)
ICD	26 (17)
Follow-up (yrs)	5.6 (1.7–11.4)
Complications	
MACE	27 (18)
HTx	14 (9)
Death	11 (7)

Values are given as n (%) or median (interquartile range). HTx, Heart transplantation, ICD, Implantable cardioverter defibrillator, LVEDD, Left ventricular end-diastolic diameter, LV, Left ventricular, LVNC, Left ventricular noncompaction cardiomyopathy, LV-EF, Left ventricular ejection fraction, MACE, Major adverse cardiac events.

TABLE 2 | Genetic findings in unrelated LVNC index patients.

	All *n* = 113
Patients with 0 variants	26 (23)
Patients with 1 variant	53 (47)
Patients with 2 variants	23 (20)
Patients with ≥3 variants	11 (10)
Patients with VUS variant	69 (61)
Patients with (likely) pathogenic variant	35 (31)
Patients with VUS only	52 (46)
Patients with (likely) pathogenic variants only	18 (16)
Patients with VUS and (likely) pathogenic variants	17 (15)
Total variants, *n*	134
Total VUS, *n*	95
Total likely pathogenic variants, *n*	24
Total pathogenic variants, *n*	15
De novo variants	
Yes	6
No	39
Unknown	89
Type of variants	
Missense, *n*	94
Frameshift, *n*	11
Stop gain, *n*	9
Splice site, *n*	17
Heterozygous variants, *n*	129
Homozygous variants, *n*	1
Hemizygous variants, *n*	4
Compound heterozygote, *n*	1

Values are given as n (%). LVNC, Left ventricular noncompaction cardiomyopathy; VUS, Variant of uncertain significance.

a hypertrabeculated myocardium without LVNC. Overall, 149 individuals with LVNC CMP were enrolled in the study at a median age of 27.8 (9.2–44.8) years. Of these 149 individuals with LVNC, 58% were symptomatic, 18% suffered from arrhythmias and 17% had an implantable cardioverter defibrillator (ICD) implanted (**Table 1**). Ventricular tachycardia occurred in 19/149 patients (13%). 55/137 patients (40%) were ≤18 years at diagnosis. Ventricular septal defect was the most common congenital heart defect in 12/149 patients (8%), and patent foramen ovale, Ebstein anomaly, patent ductus arteriosus, and other congenital heart defects were also noted (**Table 1**).

Genetic Findings in Index Patients

A total of 134 variants were identified in 87/113 (77%) index patients. Ninety-three of those variants were classified as VUS, 24 as likely pathogenic and 15 as pathogenic (**Table 2**, **Supplementary Table 1**). The genetic yield of (likely) pathogenic variants was 31% corresponding to 35/113 index patients. Missense variants ($n = 94$; 70.1%) were observed most often. Variants occurred most frequently in *MYH7* ($n = 19$), *TTN* ($n = 10$) and *MYBPC3* ($n = 8$) (**Figure 2A**). Altogether, variants in sarcomere genes constituted 42.5% ($n = 57$) of all variants (**Figure 2B**). The testing of family members for the variant found in the respective index patient revealed 78 variant carriers. 124 family members did not carry variants or were not tested (**Figure 1**).

Previously published Sanger sequencing of 8 genes in 63 patients included in this study had resulted in 18 pathogenic variants in 5 genes (26). Through bioinformatic reevaluation with current ACMG guidelines 2/18 variants were not reported in this study because the MAF was >0.0001. Through NGS, 47 additional variants were identified

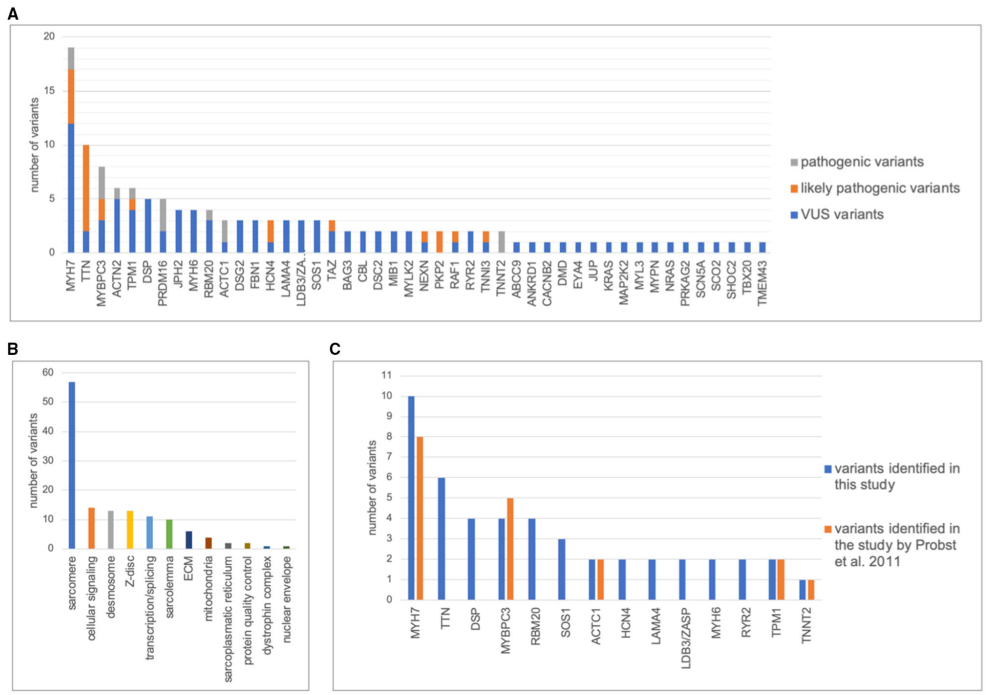

FIGURE 2 | Distribution of genetic variants. Distribution of variants in cardiomyopathy genes including pathogenic, likely pathogenic, and variants of unknown significance (VUS). The figure only contains the most frequent genes. **(A)**, Number of variants detected per gene. **(B)**, Distribution of variants between functional groups. **(C)**, Number of variants found in 63 index patients by Sanger sequencing of 8 cardiomyopathy genes by Probst et al., 2011 (26) compared with next generation sequencing of 89 cardiomyopathy genes in this study.

in 31 different genes in 43/63 patients (**Figure 2C**). Most additional variants were found in *TTN* which was not included in Sanger sequencing of the previous study. Of those additional 47 variants, 8 were classified as (likely) pathogenic. Altogether, we report a genetic yield of (likely) pathogenic variants in 16/63 (25%) patients in targeted panel sequencing compared to 18/63 (29%) patients in our previous study (26).

Genetic Variants and Phenotype

Kaplan-Meier analysis did not show differences in event-free survival between the four groups classified according to presence of genetic variants. The presence of variants in specific genes did not affect event-free survival time nor was it associated with specific phenotypes (data not shown). Between patients with variants in sarcomere genes and patients without variants in sarcomere genes no differences for the risk of MACE were found (HR: 0.73; CI 95%: 0.31–1.72).

Follow-Up

Overall, 27 events classified as MACE occurred in the study cohort during a median follow-up time of 5.6 (1.7–11.4) years. We had follow-up echocardiography data available for 89 patients. Out of those, 44% ($n = 39$) had a reduced LV systolic function at first echo and 40% ($n = 36$) at follow-up. 48% ($n = 43$) had an elevated LVEDD at first presentation and 35% ($n = 31$) at follow-up.

Echocardiography

Patients with both, increased LVEDD and reduced LV systolic function were more often symptomatic (78 vs. 43%, $p < 0.001$) and had more ICDs implanted (33 vs. 11%, $p = 0.003$) than patients without increased LVEDD and normal LV systolic function (**Table 3**). 38.3 % of patients with reduced LV systolic function at first echo underwent Htx or died during follow-up, compared to only 8.2% of patients with normal LV systolic function (**Figure 3A**). 29.2% of patients with increased LVEDD at first echo and 38.7% with the combination of increased LVEDD and reduced LV systolic function at first presentation underwent

TABLE 3 | LVNC subtypes and echocardiographic parameters.

	SUBTYPES					LVEDD AND LV SYSTOLIC FUNCTION			
	All *n* = 109	**Isolated LVNC *n* = 52 (48%)**	**Dilated LVNC *n* = 35 (32%)**	**Hypertrophic LVNC *n* =22 (20%)**	**P-value**	**All *n* = 120**	**Normal LVEDD and normal LV systolic function *n* = 81 (68%)**	**Increased LVEDD and reduced LV systolic function *n* = 39 (33%)**	***P*-value**
Female	39 (36)	21 (40)	10 (29)	8 (36)	0.529	44 (37)	34 (42)	10 (26)	0.082
Age at diagnosis (yrs)	27.2 (10.4–44.7)	26.6 (17.9–42.2)	33.6 (11.6–50.0)	1.9 (0.2–29.2)	**0.029**	28.2 (10.7–44.7)	24.3 (10.4–40.0)	38.5 (11.6–52.6)	0.092
<18 years at diagnosis	40 (40)	13 (29)	10 (31)	18 (77)	**<0.001**	46 (38)	35 (43)	11 (28)	0.113
Body surface area (m^2)	1.66 (1.15–1.92)	1.67 (1.50–1.92)	1.79 (1.53–1.99)	0.96 (0.32–1.54)	**0.001**	1.64 (1.18–1.90)	1.56 (1.09–1.86)	1.76 (1.46–1.93)	0.107
Symptomatic	54 (57)	23 (52)	22 (69)	9 (47)	0.232	61 (55)	32 (43)	29 (78)	**<0.001**
Congenital heart defect	21 (19)	10 (19)	3 (9)	8 (36)	**0.035**	20 (17)	17 (21)	3 (8)	0.067
Ventricular septal defect	10 (9)	4 (8)	1 (3)	5 (23)	0.055	9 (8)	9 (11)	0 (0)	**0.030**
Patent foramen ovale	8 (7)	4 (8)	1 (3)	3 (14)	0.318	10 (8)	7 (9)	3 (8)	1.000
Ebstein anomaly	3 (3)	1 (2)	1 (3)	1 (5)	0.779	3 (3)	3 (4)	0 (0)	0.550
Patent ductus arteriosus	4 (4)	2 (4)	1 (3)	1 (5)	1.000	4 (3)	3 (4)	1 (3)	1.000
Other congenital heart defects	5 (5)	3 (6)	1 (3)	1 (5)	0.851	4 (3)	4 (5)	0 (0)	0.303
Echocardiography									
Reduced LV systolic function	48 (45)	16 (31)	25 (74)	7 (35)	**<0.001**	56 (47)	17 (21)	39 (100)	**<0.001**
LV-EF (%)	48.0 (35.0–63.0)	54.0 (42.0–64.5)	37.0 (27.0–47.8)	58.5 (28.0–71.5)	**0.001**	46.8 (34.0–64.0)	57.5 (45.5–65.5)	31.0 (20.0–40.0)	**<0.001**
Increased LVEDD	43 (43)	1 (2)	32 (100)	10 (46)	**<0.001**	55 (46)	16 (20)	39 (100)	**<0.001**
LVEDD (mm) (patients >18 yrs only)	53.0 (49.0–65.0)	50.0 (48.0–53.0)	65.0 (61.0–75.0)	49.0 (42.0–53.0)	**<0.001**	54.5 (49.0–65.0)	50.1 (48.0–54.0)	66.0 (62.0–74.0)	**<0.001**
LVEDD Z-score (patients <18 yrs only)	1.78 (0.39–4.39)	0.05 (−0.76–0.56)	3.98 (2.35–4.56)	2.05 (0.80–4.52)	**<0.001**	1.76 (0.40–4.39)	1.04 (0.30–2.22)	5.71 (4.39–8.76)	**<0.001**
Increased LVEDD and reduced LV systolic function	30 (31)	1 (2)	24 (75)	5 (5)	**<0.001**	-	-	-	-
ECG									
ST-Depression	14 (13)	7 (14)	6 (17)	1 (5)	0.419	19 (16)	10 (12)	9 (23)	0.131
T-Inversion	14 (13)	5 (10)	5 (14)	4 (18)	0.526	18 (15)	12 (15)	6 (15)	0.935
Bundle branch block	11 (14)	4 (11)	7 (28)	0 (0)	**0.037**	18 (18)	7 (10)	11 (34)	**0.004**
Arrhythmias	19 (17)	5 (10)	10 (29)	4 (18)	0.072	23 (19)	9 (11)	14 (36)	**0.002**
Atrial fibrillation	2 (2)	1 (2)	1 (3)	0 (0)	1.000	2 (2)	1 (1)	1 (3)	0.546
Atrioventricular block II°/III°	1 (1)	1 (3)	0 (0)	0 (0)	1.000	1 (1)	1 (2)	0 (0)	1.000

(Continued)

TABLE 3 | Continued

	SUBTYPES					LVEDD AND LV SYSTOLIC FUNCTION			
	All ***n*** **= 109**	**Isolated LVNC** ***n*** **= 52 (48%)**	**Dilated LVNC** ***n*** **= 35 (32%)**	**Hypertrophic LVNC** ***n*** **=22 (20%)**	**P-value**	**All** ***n*** **= 120**	**Normal LVEDD and normal LV systolic function** ***n*** **= 81 (68%)**	**Increased LVEDD and reduced LV systolic function** ***n*** **= 39 (33%)**	***P*-value**
Supraventricular tachycardia	6 (6)	0 (0)	3 (9)	3 (14)	**0.017**	8 (7)	4 (5)	4 (10)	0.435
Ventricular tachycardia	14 (13)	4 (8)	7 (20)	3 (14)	0.213	15 (13)	5 (6)	10 (26)	**0.006**
ICD	22 (20)	6 (12)	13 (37)	3 (14)	**0.010**	22 (18)	9 (11)	13 (33)	**0.003**
Follow-up (yrs)	6.9 (2.2–11.4)	6.1 (0.8–11.3)	7.9 (3.0–12.4)	6.3 (3.5–10.5)	0.515	6.5 (2.2–11.5)	5.5 (2.0–11.1)	8.7 (2.4–15.2)	0.146
Complications									
MACE	15 (14)	4 (8)	4 (11)	7 (32)	**0.026**	22 (18)	9 (11)	13 (33)	**0.003**
HTx	7 (6)	2 (4)	2 (6)	3 (14)	0.302	11 (9)	2 (3)	9 (23)	**0.001**
Death	5 (5)	1 (2)	2 (6)	2 (9)	0.282	9 (8)	3 (4)	6 (15)	0.057

Values are given as n (%) or median (interquartile range).
HTx, Heart transplantation, ICD, Implantable cardioverter defibrillator, LVEDD, Left ventricular end-diastolic diameter, LV, Left ventricular, LVNC, Left ventricular noncompaction cardiomyopathy, LV-EF, Left ventricular ejection fraction, MACE, Major adverse cardiac events.
Values in bold indicate statistical significance.

Htx or died during follow-up (**Figures 3B,C**). Reduced LV systolic function, increased LVEDD, and a combination of both were risk factors for shorter event-free survival in the Kaplan-Meier analysis (**Figure 4**). Multivariate analysis revealed reduced LV systolic function as risk factor for event-free survival. Patients with reduced LV systolic function had 4.6 -fold higher risk for MACE (**Table 4**).

Adult Versus Pediatric Patients

The genetic variant burden of pediatric vs. adult patients can be found in **Supplementary Table 2**. Adult patients were significantly more symptomatic than pediatric patients and presented with reduced LV systolic function, had more ECG-abnormalities and a higher rate of ICDs. In the pediatric cohort we found a higher prevalence of hypertrophic LVNC (**Supplementary Table 3**). The presence or absence of variants did not correlate with the risk of developing a MACE or the event-free survival time, neither in the pediatric nor in the adult cohort. As shown in **Supplementary Table 4**, hazard ratio analysis identified lower BSA, lower LV-EF (%), increased LVEDD and the presence of symptoms as factors for a higher risk for MACE in our cohort. In adults, an older age at diagnosis increased the risk for MACE. In pediatric patients, age at diagnosis had no impact on MACE. Multivariate analysis revealed lower LV-EF as independent risk factor for MACE in the whole cohort and in the pediatric subcohort (**Supplementary Table 5**).

Pediatric Patients

In pediatric patients, a lower BSA of 0.1 m^2 increased the risk of MACE by 7.4%. The LV-EF reduction was the main risk factor with a higher independent impact than a lower BSA or increased LVEDD. The risk for MACE was decreased by approximately 8% for each additional percent of LV-EF (for comparison 4% in adults) (**Supplementary Tables 4, 5**).

Phenotypic Subtypes

We classified 109 patients into the LVNC CMP subtypes. 52 (47.7%) presented with isolated LNVC CMP, 35 (32.1%) with dilated LVNC CMP and 22 (20.2%) with hypertrophic LVNC CMP (**Table 3**). Patients with hypertrophic LVNC CMP were younger at diagnosis, more frequently affected by congenital heart defects, and at higher risk (OR: 4.61; CI 95%: 1.45–14.63) for MACE ($p = 0.01$). Patients with dilated LVNC presented more frequently with a reduced LV systolic function, had the highest rate of arrhythmias (31%), and ICDs implanted (37%). The presence of dilated LVNC CMP did not have an impact on the likelihood of MACE (OR: 0.74; CI 95%: 0.22–2.51) despite a lower LV-EF, neither did the presence of isolated LVNC (OR: 0.35; CI 95%: 0.10–1.17). The analysis of event-free survival did not show any differences between the subtypes as shown in **Supplementary Figure 1**.

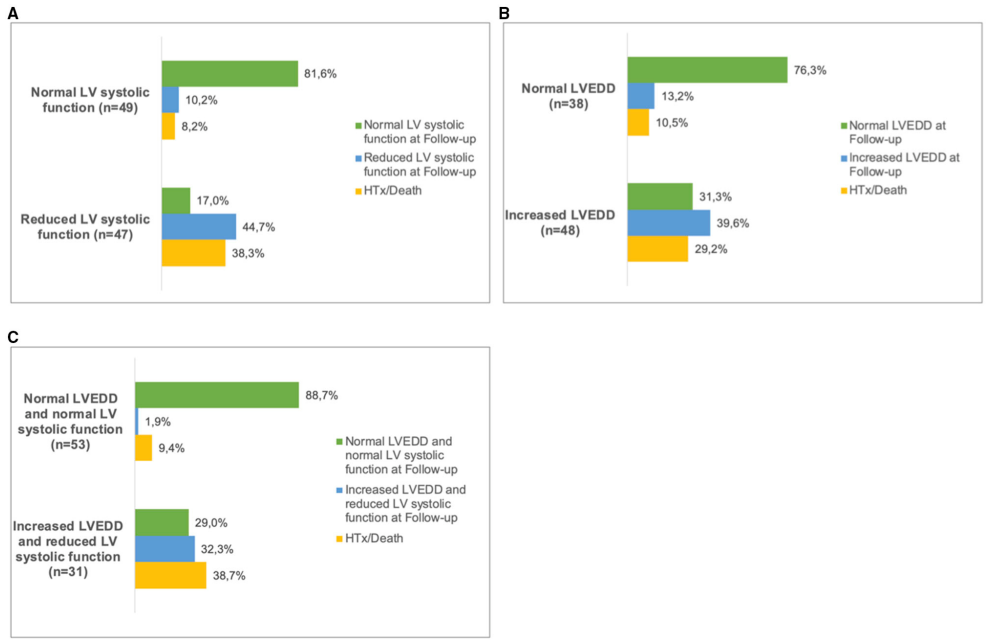

FIGURE 3 | Follow up in LVNC patients. Patients were sorted into subgroups according to their phenotype at first presentation. **(A)**, normal LV systolic function and reduced LV systolic function. **(B)**, normal LVEDD and increased LVEDD and **(C)**, 'normal LVEDD and normal LV systolic function' and 'increased LVEDD and reduced LV systolic function'. At the last available follow-up, the respective phenotypes were recorded. In some of the patients, heart transplantation (HTx) or death had occurred. LV, left ventricular; LVEDD, Left ventricular end-diastolic diameter.

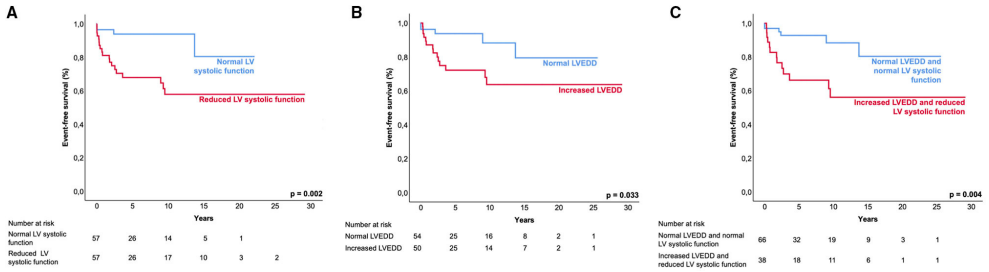

FIGURE 4 | Event-free survival of LVNC patients. Kaplan-Meier analysis shows the event-free survival to the combined endpoint of mechanical circulatory support, heart transplantation, survived sudden cardiac death, and all-cause death. Event-free survival between patient groups. **(A)**, normal LV systolic function and reduced LV systolic function; **(B)**, normal LVEDD and increased LVEDD; and **(C)**, 'normal LVEDD and normal LV systolic function' and 'increased LVEDD and reduced LV systolic function'. LV, left ventricular; LVEDD, left ventricular end-diastolic diameter.

Genetic Characteristics and Clinical Outcome

The presence or absence of (likely) pathogenic variants or variants in specific genes did not allow a risk stratification for MACE or the duration of event-free survival (data not shown). The presence of one or multiple VUS variants in addition to a (likely) pathogenic variant in a patient also failed to correlate with a higher risk for MACE

TABLE 4 | Risk for MACE.

	Univariate		Multivariate	
	HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**
Reduced LV systolic function	4.60 (1.56–13.55)	**0.006**	4.20 (1.11–15.89)	**0.035**
LV-EF (%)	0.94 (0.92–0.97)	**<0.001**	-	-
Increased LVEDD	2.89 (1.04–8.04)	**0.042**	1.62 (0.54–4.86)	0.393
Increased LVEDD and reduced LV systolic function	3.78 (1.44–9.96)	**0.007**	-	-

Dashes (-) indicate variables that were not included in multivariate analysis.

LVEDD, Left ventricular end-diastolic diameter, LV, Left ventricular, LV-EF, Left ventricular ejection fraction, MACE, Major adverse cardiac events.
Values in bold indicate statistical significance.

than only a (likely) pathogenic variant (HR: 2.17; CI 95%: 0.40–11.90).

DISCUSSION

We investigated a cohort of 113 pediatric and adult LVNC CMP patients for genetic and clinical parameters to predict outcome. We included affected and unaffected family members from 54 families. We identified reduced LV systolic function as a strong, independent risk factor for MACE. In pediatric patients, a lower BSA and lower LV-EF predicted a worse outcome. Genetic variants did not correlate with clinical outcome. Altogether, the genetic yield of (likely) pathogenic variants using targeted panel sequencing was 31%, well comparable to previous studies. Genetic screening should be focused on validated genes and is useful in family counseling.

Implications for Outcome

Echocardiography is used most commonly for diagnosis according to the Jenni criteria (1), and also seems to be the best, widely available tool for basic risk stratification.

Adult LVNC CMP patients with normal LV function were reported to have no higher mortality than the general population (8). Multivariate analysis identified age at diagnosis and LV dilatation as independent risk factors (8). Left ventricular dilation and systolic dysfunction were less strong predictors for survival than New York Heart Association class III/IV and cardiovascular complications at presentation (27). According to our results, reduced LV systolic function is the most important prognostic factor for clinical outcome (28, 29). Asymptomatic patients with normal echocardiography mostly remain with normal cardiac function during long-term follow-up. There is a clear association between symptomatic patients with abnormal echocardiographic findings and an impaired long-term clinical outcome. Previous reports described a noncompaction phenotype in pregnancy, athletes and other cardiac healthy individuals without functional impairment (30–32). In these patients, noncompaction is often reversible, does not affect cardiac function and is not associated with a CMP. Therefore, LVNC should not be labeled as a cardiomyopathy under these circumstances. The judgement of the phenotype as a disease should therefore probably rather be made by functional parameters determined by echocardiography or CMR imaging (33). In an adult cohort an association between reduced LV systolic function and mid-basal wall involvement was shown (8). Deeper phenotyping by CMR imaging showed that diffuse myocardial fibrosis contributed to heart failure in a pediatric DCM cohort and may lead to new clues in pediatric LVNC, as well (34).

Adult Versus Pediatric Patients

A systematic review of a larger LVNC cohort reported on worse clinical outcome in children (35). This was not found in our cohort and may be due to an older range of the pediatric cohort (median age 1.9 vs. 0 years) and exclusion of children with genetic syndromes, chromosomal defects, and neuromuscular symptoms. Nevertheless, lower BSA and younger age are considered risk factors for MACE. Our study showed a higher rate of asymptomatic children compared to asymptomatic adults, which might be explained by a referral bias of asymptomatic adults being sent less frequently to tertiary centers of this study. The rate of 31% asymptomatic adults was comparable to other adult cohorts (8).

The Impact of LVNC Subtypes

Based on the classification by Van Waning et al. we used 3 subgroups to classify our patients (18). Nearly half of the cohort in their study (18) and in this study were classified as isolated LVNC CMP without dilatation or hypertrophy, 42 and 48%, respectively. These findings support the general consensus defining LVNC as a distinct myocardial disease. In some cases, an overlap with other cardiomyopathies might still be suspected, especially because family members with DCM or HCM without noncompaction can also be found (18, 36). Additionally, many of the mutated genes were described causing other primary cardiomyopathies (14, 15). Meanwhile, LVNC-specific variants probably explain 5–10% of cases (16). It has been shown, that pediatric patients with isolated LVNC CMP have the best outcome compared to patients with LVNC and an underlying DCM/HCM (5). One might suspect an overlap with noncompaction without cardiomyopathy, like it has been discussed before (33).

Genotype-Phenotype Correlation

Mutations in *MYH7*, *TTN* and *MYBPC3* were most prevalent in our study, as described by others (35, 37). The evidence

for genotype-phenotype correlations remains controversial (4, 38). Nevertheless, with the focus on an impaired LV systolic function of pediatric patients with LVNC CMP, van Waning et al. suggest that including genetic findings in children may be helpful predicting clinical outcome and may be appropriate in clinical management (4). On the other hand, genetic counseling is recommended, for young patients and valuable for family counseling (35).

Variants in specific genes were associated with worse outcome in LVNC, as reported for variants in *Lamin A/C*, *RBM20*, *TAZ*, Titin-truncating variants and non-sarcomere genes in general (13, 37, 39, 40). Overall, larger cohorts, and genotype-phenotype studies analyzing the correlation between genetic background and clinical outcome are needed in the future. Based on these findings more patient-individual genetic counseling and more precise disease management becomes possible.

Family Screening

Potential non-penetrance of variants, as described in systematic family screening of pediatric primary cardiomyopathies before (41), might be a reason for asymptomatic variant carriers identified through family screening. One possible explanation for intra-familial variability might be the role of genetic modifiers.

Limitations

Our cohort was heterogenous and consisted of patients from different hospitals. Clinical data was collected from medical reports from different physicians and an information bias cannot be ruled out. Also, genetic and clinical data on family members was not available for many index patients. Clinical data from adults and children cannot always be directly compared. Therefore, we converted numerical data into dichotomous variables. Our limited cohort size led to small subgroups, which limited the statistical power. Especially in the pediatric cohort, syndromic comorbidities and other heart defects may not always be identified or reported. A referral bias of more severe cases is possible.

CONCLUSIONS

We performed a retrospective study on a large cohort of LVNC CMP patients to determine genetic and clinical factors to predict the clinical course and outcome of LVNC. We report that reduced LV systolic function is a risk factor for MACE in pediatric patients and in adults. The presence or absence of genetic variants was not predictive for the risk of developing a MACE, neither in the pediatric nor in the adult cohort. Genetic screening is useful for cascade screening to identify family members at risk for developing LVNC CMP.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Charite Universitätsmedizin Berlin, Berlin, Germany. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AS-B and SK contributed to conception, design of the study, and wrote the first draft of the manuscript. WK, RJ, MG, EO, and FB contributed patient data. AS-B, JK, CH, FS, NA-W-M, JD, and ST analyzed clinical and genetic data and organized the database. AS-B performed the statistical analysis. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.722926/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Komplette Publikationsliste

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