



# Pathogenic Variants Associated With Dilated Cardiomyopathy Predict Outcome in Pediatric Myocarditis

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**BACKGROUND:** Myocarditis is one of the most common causes leading to heart failure in children and a possible genetic background has been postulated. We sought to characterize the clinical and genetic characteristics in patients with myocarditis  $\leq 18$  years of age to predict outcome.

**METHODS:** A cohort of 42 patients (Genetics in Pediatric Myocarditis) with biopsy-proven myocarditis underwent genetic testing with targeted panel sequencing of cardiomyopathy-associated genes. Genetics in Pediatric Myocarditis patients were divided into subgroups according to the phenotype of dilated cardiomyopathy (DCM) at presentation, resulting in 22 patients without DCM (myocarditis without phenotype of DCM) and 20 patients with DCM (myocarditis with phenotype of DCM).

**RESULTS:** Myocarditis with phenotype of DCM patients (median age 1.4 years) were younger than myocarditis without phenotype of DCM patients (median age 16.1 years;  $P < 0.001$ ) and were corresponding to heart failure-like and coronary syndrome-like phenotypes, respectively. At least one likely pathogenic/pathogenic variant was identified in 9 out of 42 patients (22%), 8 of them were heterozygous, and 7 out of 9 were in myocarditis with phenotype of DCM. Likely pathogenic/pathogenic variants were found in genes validated for primary DCM (*BAG3*, *DSP*, *LMNA*, *MYH7*, *TNNI3*, *TNNT2*, and *TTM*). Rare variant enrichment analysis revealed significant accumulation of high-impact disease variants in myocarditis with phenotype of DCM versus healthy individuals ( $P = 0.0003$ ). Event-free survival was lower ( $P = 0.008$ ) in myocarditis with phenotype of DCM patients compared with myocarditis without phenotype of DCM and primary DCM.

**CONCLUSIONS:** We report heterozygous likely pathogenic/pathogenic variants in biopsy-proven pediatric myocarditis. Myocarditis patients with DCM phenotype were characterized by early-onset heart failure, significant enrichment of likely pathogenic/pathogenic variants, and poor outcome. These phenotype-specific and age group-specific findings will be useful for personalized management of these patients. Genetic evaluation in children newly diagnosed with myocarditis and DCM phenotype is warranted.

**Key Words:** biopsy, endomyocardial ■ cardiomyopathy, dilated ■ genetics ■ myocarditis

Myocarditis is a common cause of childhood heart failure.<sup>1</sup> Although myocarditis and idiopathic dilated cardiomyopathy (DCM) are considered distinct entities, myocarditis frequently presents with a

phenotype of new-onset DCM.<sup>2</sup> The diagnosis is challenging due to the heterogeneity of clinical presentations.<sup>3</sup> A definite diagnosis requires endomyocardial biopsy (EMB), which is often still not part of routine

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## Nonstandard Abbreviations and Acronyms

<b>EMB</b>	endomyocardial biopsy
<b>HTx</b>	Heart transplantation
<b>LP/P</b>	likely pathogenic/pathogenic
<b>MYC-DCM</b>	myocarditis with phenotype of dilated cardiomyopathy
<b>MYC-NonDCM</b>	myocarditis without phenotype of dilated cardiomyopathy
<b>MYCPEDIG</b>	Genetics in Pediatric Myocarditis
<b>RIKADA</b>	Risk Stratification in Children and Adolescents With Primary Cardiomyopathy
<b>RIKADA-DCM</b>	DCM cohort of the study RIKADA

practice.<sup>4</sup> A marked disparity has been reported in outcomes in children with a new-onset DCM phenotype associated with myocarditis compared to those with idiopathic DCM.<sup>5,6</sup> Specifically, pediatric patients with myocarditis had lower rates of heart transplantation (HTx) and death compared to individuals with idiopathic DCM.<sup>7</sup> This implicates that differentiating these diseases is an important part of the initial diagnostic and treatment plan.<sup>5–8</sup>

Evidence that viral infection can influence the severity and penetrance of DCM has long been recognized.<sup>9</sup> A molecular mechanism through which enteroviral infection contributes to the pathogenesis of secondary forms of DCM was identified.<sup>10</sup> Dystrophin deficiency in mice markedly increased enterovirus-induced DCM.<sup>11</sup> Recently, it has been shown that 12% of children with acute myocarditis carried pathogenic autosomal recessive variants in cardiac disease genes.<sup>12</sup> It was proposed that genetic defects in structural proteins may cause the myocardium to become vulnerable and predisposed to myocardial inflammation with mutation in genes associated with arrhythmogenic cardiomyopathy (AC) or arrhythmogenic right ventricular cardiomyopathy (ARVC).<sup>13,14</sup> In adult myocarditis, in association with AC/ARVC, pathogenic variants in desmoplakin (*DSP*) were identified in small case series.<sup>14–17</sup> Most of the studies included cases of suspected myocarditis, some cases were reported in children, and myocarditis was rarely biopsy-proven. In none of these studies, age group-specific genetic findings were reported. Also, no study systematically divided the cohort into subgroups according to the presence or absence of DCM at presentation.

The objectives of the present analysis, therefore, were to (1) determine pathogenic genetic variants in a cohort of EMB-proven pediatric myocarditis, (2) assess clinical and genetic differences in pediatric myocarditis with and without DCM phenotype, and (3) explore the possible value of genetic diagnosis for outcome in pediatric myocarditis. We hypothesized that children with definite

myocarditis would have pathogenic variants in structural cardiac genes predicting acute or chronic cardiac dysfunction and clinical outcome.

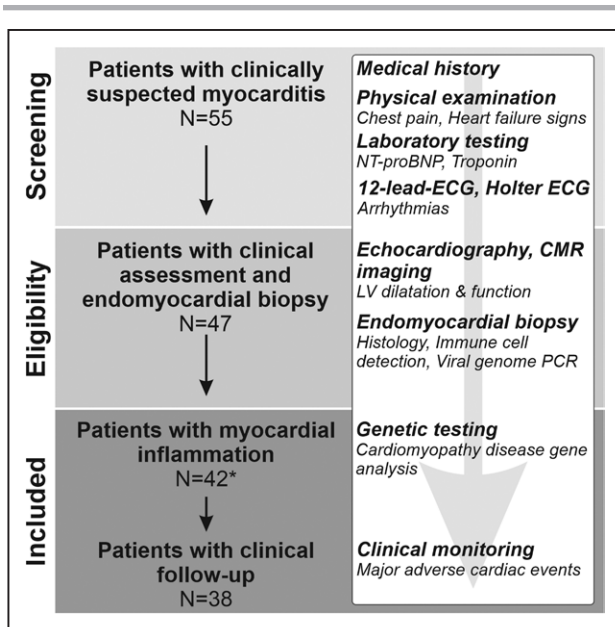
## METHODS

Study methods can be found in the [Data Supplement](#). The study was approved by the institutional ethics committee Charité - Universitätsmedizin Berlin following the Declaration of Helsinki. All parents/guardians of patients <18 years gave written informed consent. The data that support the findings of this study are available from the corresponding author upon reasonable request. A subset of the data generated for this study are available at the ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar/>).<sup>18</sup>

## RESULTS

### Clinical Characterization Genetics in Pediatric Myocarditis Cohort

The Genetics in Pediatric Myocarditis (MYCPEDIG) cohort was composed of patients with biopsy-proven myocarditis who underwent clinical and diagnostic assessments including laboratory parameters and cardiac imaging (Figure 1). The major clinical characteristics of the MYCPEDIG cohort are presented in Table 1.<sup>19,20</sup> Forty-two unrelated patients with biopsy-proven myocarditis and a median age at diagnosis of 10.0 (interquartile range, 1.1–16.4) years were included in the study. A total of 17 patients were female and 25 were male. All individuals underwent the same standardized genetic analysis with targeted panel sequencing. The cohort was divided into 2 subgroups, patients presenting with DCM phenotype (myocarditis with phenotype of DCM [MYC-DCM], n=20) and patients without DCM phenotype (myocarditis without phenotype of DCM [MYC-NonDCM], n=22; Table 1). The subgroup MYC-DCM contained significantly younger patients with a median age of 1.4 (interquartile range, 0.3–4.1) years compared to the MYC-NonDCM patients with a median age of 16.1 (interquartile range, 11.5–17.1) years ( $P<0.001$ ). MYC-DCM patients more often presented with higher New York Heart Association classes ( $P<0.001$ ), dyspnea ( $P=0.005$ ), gastrointestinal symptoms ( $P=0.018$ ), and signs of cardiac decompensation ( $P=0.001$ ). The MYC-NonDCM subgroup was characterized by higher frequency of chest pain ( $P<0.001$ ) and fever within the last 6 weeks before admission ( $P=0.011$ ). Troponin was elevated in both subgroups without statistical difference, with 86% in the MYC-NonDCM and 81% in the MYC-DCM subgroup. Further characteristics of the 2 subgroups in laboratory findings, ECG, echocardiography, CMR, and medication are listed in Table 1. In-depth phenotyping revealed that these subgroups were corresponding to coronary syndrome-like (MYC-NonDCM) and heart failure-like phenotype (MYC-DCM).



**Figure 1. Study flow chart.**

Study design of the Genetics in Pediatric Myocarditis cohort, including only patients with biopsy-proven myocarditis, the performed diagnostics, and follow-up. CMR indicates cardiovascular magnetic resonance imaging; LV, left ventricular; NT-proBNP, N-terminal pro-brain natriuretic peptide; and PCR, polymerase chain reaction. \*In 2 patients with a previous diagnosis of primary dilated cardiomyopathy, myocarditis was subsequently proven by endomyocardial biopsy (EMB).

All patients presented with lymphocytic myocarditis (Table I in the [Data Supplement](#)). The MYC-DCM subgroup showed higher prevalence of necrosis ( $P=0.04$ ) and mononuclear cell infiltrates ( $P=0.007$ ). Specifically, the CD3+ lymphocyte or CD68+ macrophage counts were significantly elevated in the MYC-DCM subgroup ( $P=0.003$  and  $0.002$ , respectively). Viral genome detection was 29% in the MYC-NonDCM subgroup and 58% in the MYC-DCM subgroup and did not differ between the two subgroups ( $P=0.109$ ). Thus, MYC-DCM patients had more severe signs of inflammation compared to the MYC-NonDCM patients.

## Genetic Analysis

Within the MYCPEDIG cohort, we identified 9 likely pathogenic/pathogenic (LP/P) variants in 42 (22%) patients (Table 2). Pathogenic variants were detected in 2 patients, LP variants in 7 patients, and variant of uncertain significance in 13 patients. Eight of 9 LP/P variants were heterozygous, and one variant was homozygous. Seven out of 20 (35%) LP/P variants were in MYC-DCM, and 2 out of 22 (9%) were in MYC-NonDCM ( $P=0.062$ ). None of the patients carried 2 LP/P variants. Details of individual variants, classification according to The American College of Medical Genetics and Genomics (ACMG), complex genotypes, classification with myocarditis subgroup,

and the genetic family context are available in the [Data Supplement](#) (Table II through IV in the [Data Supplement](#)). Functional clustering revealed LP/P variants most frequently in genes associated with sarcomere, Z-Disc, and desmosome function. In a patient with the BAG cochaperone 3 (*BAG3*) LP frameshift variant p.Tyr205Thrfs\*6 reduced BAG3 protein levels and a disturbed sarcomere organization were found in EMB (Figure II in the [Data Supplement](#)).

To further evaluate the findings of the MYCPEDIG cohort, we compared the MYC-DCM and MYC-NonDCM subgroups with primary DCM patients from the RIKADA (Risk Stratification in Children and Adolescents With Primary Cardiomyopathy) cohort (RIKADA-DCM [DCM cohort of the study RIKADA]).<sup>21</sup> The main clinical characteristics of RIKADA-DCM patients are available in Table V in the [Data Supplement](#). Four RIKADA-DCM patients revealed an LP/P variant (Tables II, VI, and Table VII in the [Data Supplement](#)).

To validate our genetic findings in a control cohort, we selected the data sets from 503 healthy individuals of European descent from the International Genome Sample Resource (IGSR) data depository and performed automatic annotation followed by enrichment analysis (Figure 2). After filtering for variants in 89 cardiomyopathy-associated (CMP) genes, 264 IGSR and 39 MYC-NonDCM/MYC-DCM/RIKADA-DCM heterozygous rare variants were classified according to their Combined Annotation Dependent Depletion (CADD) score (Figure 2A). Nonparametric testing of the CADD score distribution revealed no significant difference of the medians (23.65 IGSR, 25.30 MYC-NonDCM/MYC-DCM/RIKADA-DCM,  $P=0.058$ ; Figure 2B). Applying a threshold of CADD >30, indicative for high-impact disease variants, identified 27 variants in the IGSR and 9 variants in the MYC-NonDCM/MYC-DCM/RIKADA-DCM cohort. The MYC-NonDCM/MYC-DCM/RIKADA-DCM cohort showed a significant relative enrichment of rare variants with CADD >30 compared with healthy IGSR individuals (Wilcoxon test  $P=0.0023$ , Fisher  $P=0.007$ ). Comparative analysis for enrichment of CADD >30 variants in respective subgroups revealed significant accumulation of high-impact disease variants in the MYC-DCM compared with IGSR individuals ( $P=0.0003$ ; Figure 2C). In addition, also the combined myocarditis group MYC-DCM/MYC-NonDCM and MYC-DCM/RIKADA-DCM showed significant accumulation of high-impact disease variants. No significant enrichment of CADD >30 variants was observed in the MYC-NonDCM and RIKADA-DCM cohort compared with IGSR individuals. Moreover, we performed gene-based burden testing for the MYC-DCM, MYC-NonDCM, and RIKADA-DCM subgroups. This burden analysis identified significant enrichment of truncating variants in *DSP* and *BAG3* for the MYC-NonDCM and MYC-DCM subgroups, respectively (Figure III in the [Data Supplement](#)).

**Table 1. Clinical Characteristics**

	All	MYC-NonDCM	MYC-DCM	P value
General patient parameter				
Patients	42	22	20	
Female individuals	17 (41)	8 (36)	9 (45)	0.754
Median age, y	10.0 (1.1 to 16.4)	16.1 (11.5 to 17.1)	1.4 (0.3 to 4.1)	<0.001*
BSA, kg/m <sup>2</sup>	1.2 (0.5 to 1.9)	1.8 (1.3 to 2.0)	0.6 (0.3 to 0.7)	<0.001*
Follow-up time (month)	16.1 (7.1 to 41.0)	8.0 (3.3 to 17.2)	32.0 (17.3 to 43.2)	<0.001*
Symptoms				
NYHA class I	15 (36)	14 (64)	1 (5)	<0.001*
NYHA class II	8 (19)	4 (18)	4 (20)	
NYHA class III	1 (2)	0 (0)	1 (5)	
NYHA class IV	12 (29)	3 (14)	9 (45)	
NYHA n.a.	6 (14)	1 (5)	5 (25)	
Chest pain	18 (43)	16 (73)	1 (5)	<0.001*
Chest pain n.a.	3 (7)	0 (0)	6 (30)	
Dyspnea	18 (43)	6 (27)	12 (60)	0.005*
Fatigue	37 (88)	18 (86)	18 (90)	1.000
Feeding intolerance	9 (21)	2 (9)	7 (35)	0.065
Gastrointestinal symptoms	8 (19)	1 (5)	7 (35)	0.075
Decompensation	18 (43)	4 (18)	14 (70)	0.001*
Infection (<6 wk)	21 (50)	14 (64)	7 (35)	0.121
Fever (<6 wk)	15 (36)	12 (55)	3 (15)	0.011*
ECG				
ST-elevation	20 (48)	16 (73)	4 (20)	0.001*
T-inversion	15 (36)	9 (41)	6 (30)	0.531
Arrhythmias†	16 (38)	11 (50)	5 (20)	0.121
Laboratory				
NT-proBNP, pg/mL	6465.5 (235.3 to 26358.0)	406.0 (137.5 to 2679.0)	24198.0 (8294.0 to 53520.0)	0.001*
Troponin Ihs, pg/mL	386.1 (137.1 to 1736.2) N=10	909.6 (38.6 to 1704.8) N=4	386.1 (243.7 to 11209.3) N=6	0.610
Troponin Ths, ng/L	556.0 (49.0 to 1162.0) N=25	565.0 (493.0 to 1516.0) N=15	191.5 (11.0 to 771.5) N=10	0.129
Echocardiography				
Z score LVIDD, mm	3.7 (0.1 to 5.7)	0.3 (−0.8 to 1.6)	5.9 (4.4 to 7.8)	<0.001*
LVEF, %	39.5 (22.0 to 60.0)	59.0 (55.0 to 64.3)	22.0 (18.3 to 33.8)	<0.001*
CMR‡				
	N=23	N=19	N=5	
LVEDVi, mL/m <sup>2</sup>	86.5 (74.0 to 109.0)	78.6 (64.3 to 94.8)	149.5 (117.8 to 167.0)	0.001*
LVEF, %	55.0 (39.0 to 64.0)	56.0 (53.3 to 65.5)	23.0 (16.5 to 34.5)	<0.001*
Edema	11 (48)	10 (56)	1 (20)	0.317
LGE positive	17 (74)	14 (78)	3 (60)	0.576
Medication				
Heart failure medication	34 (81)	14 (64)	20 (100)	0.004*
Inotropic medication	19 (45)	4 (18)	15 (75)	<0.001*
MCS and complications				
MCS	14 (33)	2 (9)	12 (60)	0.001*
Weaned from MCS	7 (50)	1 (50)	5 (42)	0.437
Resuscitation	8 (19)	1 (5)	7 (35)	0.018*
HTx	6 (14)	0 (0)	6 (30)	0.007*
Death	4 (10)	2 (9)	2 (10)	1.000

If not otherwise stated values are given as n (%) or median (interquartile range). BSA indicates body surface area; CMR, cardiovascular magnetic resonance imaging; DCM, dilated cardiomyopathy; HTx, heart transplantation; LGE, late gadolinium enhancement; LVEDVi, indexed left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVIDD, left ventricular end-diastolic diameter; MCS, mechanical circulatory support; MYC-DCM, myocarditis with phenotype of dilated cardiomyopathy; MYC-NonDCM, myocarditis without phenotype of dilated cardiomyopathy; n.a., not applicable; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; Troponin Ihs, Troponin I measured in high sensitivity; and Troponin Ths, Troponin T measured in high sensitivity.

\*Statistically significant values ( $P < 0.05$ ).

†Arrhythmias were recorded with ECG or Holter-ECG and contained AV block II/III, nonsustained ventricular tachycardia, supraventricular tachycardia.

‡CMR criteria used to diagnose myocardial inflammation were in accordance with the initial and revised Lake Louise criteria.<sup>19,20</sup>

**Table 2. Pathogenic and Likely Pathogenic Genetic Variants**

Variant ID	Gene	Transcript	cDNA position	Protein position	Genotype	Consequence	gnomAD frequency	Pathogenicity
CMP-100-01†	<i>BAG3</i>	ENST00000369085, NM_004281.3	c.608delG	p.Tyr205Thrfs*6	het	frameshift, protein truncation	0	LP
CMP-99-01†	<i>BAG3</i>	ENST00000369085, NM_004281.3	c.925C>T	p.Arg309*	het	stop gained	0	P
CMP-105-01‡	<i>DSP</i>	ENST00000379802, NM_004415.2	c.2200A>del	p.Arg734Glufs*31	het	frameshift, protein truncation	0	LP
CMP-81-02‡	<i>DSP</i>	ENST00000379802, NM_004415.2	c.4372C>T	p.Arg1458*	het	stop gained	0.000004	LP
CMP-89-01†	<i>LMNA</i>	ENST00000368300, NM_005572.3	c.868G>A	p.Glu290Lys	het	missense	0	P
CMP-87-01†	<i>MYH7</i>	ENST00000355349.3, NM_000257.2	c.644C>T	p.Thr215Ile	het	missense	0	LP
CMP-84-01†	<i>TNNI3</i>	ENST00000344887.5, NM_000363.4	c.204delG	p.Arg68Argfs*9	hom	frameshift, protein truncation	0.000038	P
CMP-83-03†	<i>TNNT2</i>	ENST00000455702.1, NM_001276345.1	c.460C>T	p.Arg154Trp	het	missense	0.000036	LP
CMP-90-01†	<i>TTN</i>	ENST00000342992, NM_133378.4	c.25889_25892del	p.E8630Gfs*28	het	frameshift, protein truncation	0.00003	LP

CMP indicates cardiomyopathy; gnomAD, The Genome Aggregation Database; Het, heterozygous; Hom, homozygous; LP, likely pathogenic; MYC-DCM, myocarditis with phenotype of dilated cardiomyopathy; MYC-NonDCM, myocarditis without phenotype of dilated cardiomyopathy; and P, pathogenic.

\*Represents termination codon.

†MYC-DCM.

‡MYC-NonDCM.

## Outcome

MYCPEDIG patients were followed for a median time of 16.1 (interquartile range, 7.1–41.0) months. The follow-up time and overall mortality were not different between the 2 subgroups MYC-DCM and MYC-NonDCM (Table 1). According to their clinical symptoms, patients of the MYC-DCM subgroup were more frequently resuscitated ( $P=0.018$ ), had higher need for mechanical circulatory support ( $P=0.001$ ), and underwent HTx more often ( $P=0.007$ ) compared with the MYC-NonDCM subgroup (Table 1). At follow-up, 91% (20/22) in the MYC-NonDCM subgroup presented without DCM (Figure 3). In contrast, at follow-up only 40% ( $n=8$ ) in the MYC-DCM subgroup presented without DCM, 30% ( $n=6$ ) still had DCM, and 30% had been transplanted or had died (HTx:  $n=5$ ; death:  $n=1$ ; HTx and death:  $n=1$ ; Figure 3).

In the MYCPEDIG cohort, the overall event-free survival of the combined end point mechanical circulatory support, HTx, or death was 54% after 5 years (Figure 4A). Sex had no significant impact on the occurrence of the combined end point ( $P=0.458$ ; Figure 4B). The highest event-free survival was in the MYC-NonDCM subgroup with 85%, followed by the RIKADA subgroup with 55%, and the MYC-DCM subgroup with 32% ( $P=0.008$ ; Figure 4C).

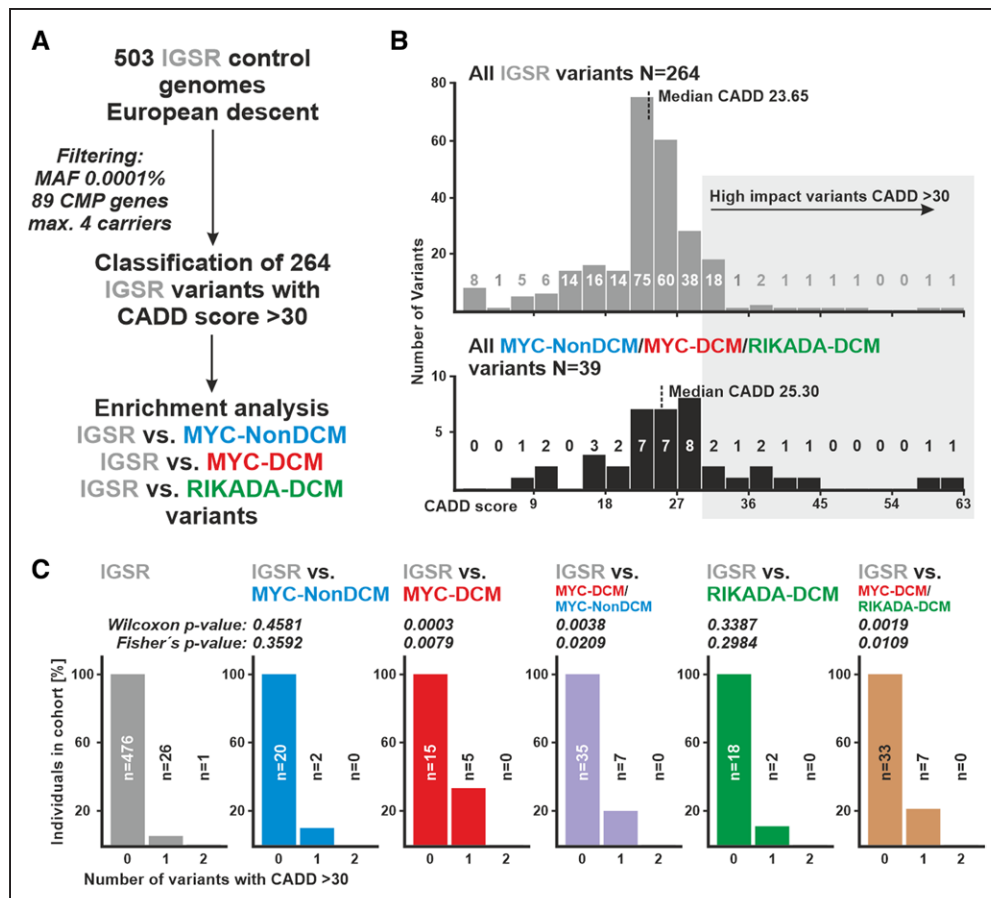
## DISCUSSION

With this study, we systematically evaluated pathogenic variants in CMP genes in EMB-proven pediatric myocarditis with implications for clinical outcome. A unique feature of our study is that all patients underwent a standard regimen of EMB-proven diagnosis and genetic evaluation. According to the presence of

a DCM phenotype, the MYCPEDIG cohort was divided into a subgroup without DCM (MYC-NonDCM) and a subgroup with DCM (MYC-DCM), respectively. In-depth phenotyping revealed that these subgroups were corresponding to coronary syndrome–like (MYC-NonDCM) and heart failure–like phenotype (MYC-DCM). Patients with a heart failure–like phenotype were significantly younger compared to patients with a coronary syndrome–like phenotype. At least one pathogenic genetic variant was identified in 9 out of 42 patients (22%), 8 of them were heterozygous. The yield of LP/P variants was substantially higher in MYC-DCM (35%) compared to MYC-NonDCM (9%). Genetic disease variants with a CADD score  $>30$  were significantly enriched in the MYC-DCM but not the MYC-NonDCM cohort compared to IGSR control individuals. Event-free survival was lower in MYC-DCM patients compared to MYC-NonDCM and primary DCM. We suggest that heterozygous DCM causing genetic variants are critical to predict outcome in myocarditis. These phenotype-specific and age group–specific findings will be useful for personalized management of these patients.

## Genetic Predisposition to Myocarditis

Myocarditis accounts for 30% to 35% of children with DCM phenotypes in pediatric CMP registries.<sup>5,6</sup> The incidence of pediatric CMP is significantly higher in the first year of life than at older ages.<sup>22</sup> We show that myocarditis with a DCM phenotype (MYC-DCM; median age 1.4 years) peaks around infancy, which is comparable to overall pediatric CMP.<sup>22</sup> It has remained unclear if children with primary CMP are misdiagnosed with myocarditis, or whether genetic variants increase

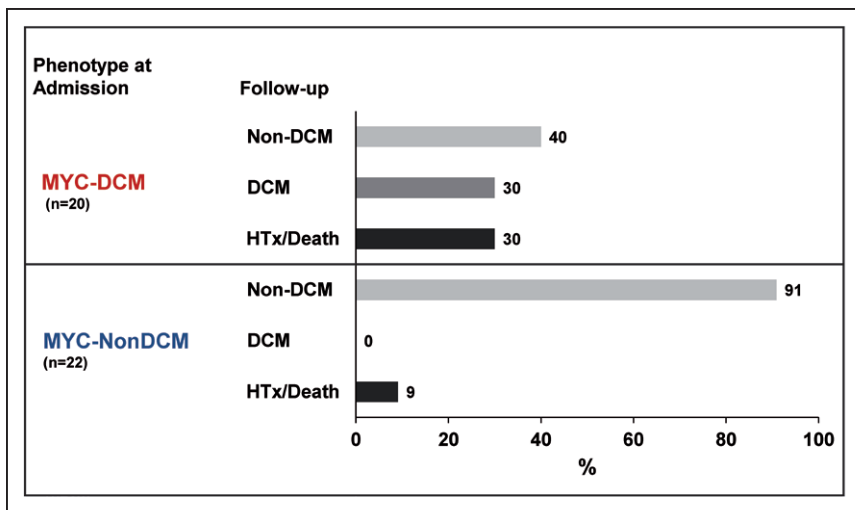


**Figure 2. Accumulation of high-impact disease variants in pediatric myocarditis.**

**A**, Enrichment of high-impact disease variants with a Combined Annotation Dependent Depletion (CADD) score >30 was analyzed in myocarditis without phenotype of dilated cardiomyopathy (MYC-NonDCM), myocarditis with phenotype of dilated cardiomyopathy (MYC-DCM), RIKADA-DCM (DCM cohort of the study Risk Stratification in Children and Adolescents With Primary Cardiomyopathy) patients compared to healthy controls from the International Genome Sample Resource (IGSR) data respiratory. IGSR controls were of European descent. Genetic variants detected in 89 cardiomyopathy (CMP) disease genes were filtered with a minor allele frequency (MAF) of 0.0001%. **B**, Classification of automatically filtered heterozygous variants was performed according to their CADD score of MYC-NonDCM/MYC-DCM/RIKADA-DCM patients and IGSR control individuals. High-impact variants with a CADD score >30 are highlighted with a gray background. **C**, Enrichment of CADD >30 variants was tested for MYC-NonDCM, MYC-DCM, MYC-DCM/MYC-NonDCM, RIKADA-DCM, and MYC-DCM/RIKADA-DCM groups compared to the IGSR cohort with the Wilcoxon rank-sum test and Fisher exact test. Significant enrichment was observed in the MYC-DCM, MYC-DCM/MYC-NonDCM, and MYC-DCM/RIKADA-DCM subgroups.

a child's susceptibility to myocarditis. In the MYC-DCM subgroup, we do not know whether and how long this phenotype or condition existed before admission. Was the DCM phenotype preexistent before myocarditis or did we see a secondary deterioration due to the inflammation, viral or nonviral triggered? Four of the detected 9 LP/P variants in our study were in *DSP* (twice) and *BAG3* (twice). Potentially pathogenic variants in desmosomal genes (*DSP*, *PKP2*) and in the thin filament gene troponin I (*TNNI3*) were found in children with suspected myocarditis.<sup>12</sup> In addition, alterations in *BAG3*, encoding a key modulator of autophagy and protein homeostasis, were associated with myocarditis in the same study. *DSP* mutation may cause advanced DCM with the pathological characteristics in explanted organs undergoing cardiac transplantation.<sup>23</sup> Obviously, variants in *DSP* cause a unique form of CMP with left ventricular (LV) phenotype and may increase

the susceptibility to superimposed acute myocarditis in ARVC.<sup>15,16,24–26</sup> Episodic myocardial injury in *DSP*-CMP contributes to disease progression and precedes systolic dysfunction.<sup>17,27</sup> The 2 *DSP* truncating variants were found in MYC-NonDCM and were the only LP/P variants in this older pediatric subgroup. This finding further suggests *DSP* to be responsible for a distinct clinical phenotype described in connection with AC/ARVC and coronary syndrome-like myocarditis in adults.<sup>14–17</sup> In a recent report, 56% of patients with suspected myocarditis and the additional features, sustained ventricular arrhythmias or RV abnormalities carried an LP/P variant, predominantly in ARVC-associated genes.<sup>14</sup> Comparable to our study, none of these patients had a history of CMP before myocarditis. Our genetic study of unselected pediatric patients with lymphocytic myocarditis showed a lower overall genetic yield (22%) with LP/P variants in DCM-associated genes.



**Figure 3. Outcome in a cohort of childhood biopsy-proven myocarditis (Genetics in Pediatric Myocarditis [MYCPEDIG]).**

Patients from the MYCPEDIG cohort were subdivided into myocarditis with phenotype of dilated cardiomyopathy (MYC-DCM) and myocarditis without phenotype of dilated cardiomyopathy (MYC-NonDCM) groups according to the phenotype at time of admission. Phenotype at follow-up was recorded, with DCM or without DCM (NonDCM). Patients receiving or heart transplantation (HTx) or died were listed separately (HTx/death).

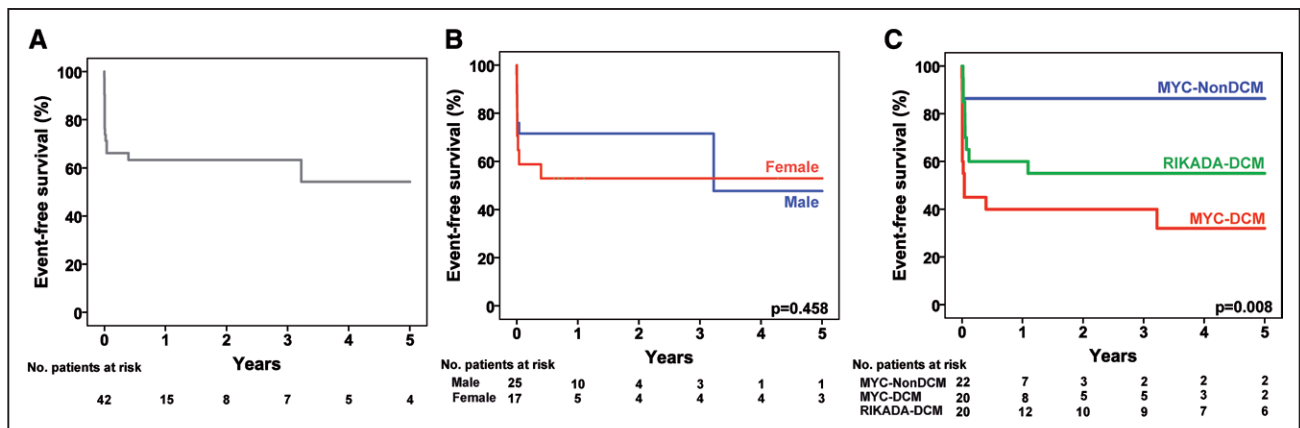
### Genetic Screening in Pediatric Myocarditis

We systematically screened pediatric EMB-proven myocarditis patients for defects in CMP disease genes. The detected LP/P variants, which appeared most frequently in a heterozygous state, were detected in genes validated for primary DCM.<sup>28,29</sup> Of note, there is complete overlap of the 12 genes described by Mazzarotto et al<sup>30</sup> as having robust disease association with DCM, and the genes with heterozygous LP/P variants in MYCPEDIG and RIKADA subgroups. In addition, in our previous analysis, we could show that no TNNI3 (Troponin I3, cardiac type) protein is detectable in heart tissue of patients with this homozygous TNNI3 truncation.<sup>31</sup> We identified one heterozygous LP variant in titin (*TTN*), a gene previously not associated with myocarditis, but considered to be a major disease gene in adult DCM.<sup>28,29</sup> In suspected acute viral myocarditis, mostly not biopsy-proven, rare recessive or compound heterozygous alleles altering genes previously associated with typically dominant genetic CMP may underlie myocarditis.<sup>12</sup> In another study,

dominant as well as recessive inheritance was observed in some cases of acute pediatric-onset heart failure.<sup>32</sup> Interestingly, the more pronounced inflammatory infiltrates within the EMB of the MYC-DCM subgroup compared to MYC-NonDCM were present without concomitant fibrosis, which would be a hallmark of primary DCM. Of note, significant relative enrichment of rare variants with CADD >30 compared with healthy IGSR individuals in our study was found in MYC-DCM but not MYC-NonDCM patients. Moreover, the difference in LP/P yields between the 2 subgroups are substantial, although statistically not significant due to the small sample sizes. This underlines the strong genetic impact of heterozygous DCM variants for early-onset, severe MYC-DCM in children. The diagnostic value of genetic testing seems high in this specific subgroup of patients.

### Outcome and Implications for Treatment

In our study, the event-free survival of the MYC-DCM subgroup was lower than in patients with primary DCM



**Figure 4. Event-free survival of the Genetics in Pediatric Myocarditis (MYCPEDIG) and RIKADA-DCM (DCM cohort of the study Risk Stratification in Children and Adolescents With Primary Cardiomyopathy) cohorts.**

Kaplan-Meier curves illustrate the event-free survival to the combined end point of death, heart transplantation, and mechanical circulatory support. Event-free survival (**A**) in the overall MYCPEDIG cohort, (**B**) between female (red) and male patients (blue;  $P=0.458$ , log-rank test), and (**C**) between the myocarditis without phenotype of dilated cardiomyopathy (MYC-NonDCM), RIKADA-DCM, and myocarditis with phenotype of dilated cardiomyopathy (MYC-DCM) subgroups ( $P=0.008$ , log-rank test).

(RIKADA-DCM). Reported outcomes and prognostic factors for childhood DCM vary considerably. The Pediatric Cardiomyopathy Registry and the National Australian Childhood Cardiomyopathy Study<sup>5,6,8</sup> established that children with a new-onset DCM phenotype have a better prognosis if they are diagnosed with myocarditis than with idiopathic DCM. Congestive heart failure is severe among children with DCM, and lymphocytic myocarditis is an important cause. Although early mortality in myocarditis is high, the clinical status of long-term survivors is good.<sup>6</sup> In children with myocarditis who had impaired LV ejection fraction at presentation, rates of echocardiographic normalization were greater in those without LV dilatation.<sup>7</sup> MYC-DCM patients had LV dysfunction and LV dilatation, fulfilling all DCM echocardiographic criteria. This pediatric subgroup of patients with myocarditis (MYC-DCM) might be distinct from adult patients with acute myocarditis that typically present with LV dysfunction but without LV dilatation.<sup>33</sup> Therefore, we can only speculate that primary DCM was present in these children with <2 years of age before myocarditis of whatever cause occurred and lead to myocardial decompensation and heart failure.

Our findings suggest that MYC-DCM patients have a higher risk for mechanical circulatory support or HTx compared with individuals with myocarditis presenting without DCM phenotype. A risk group of children with myocarditis <2 years of age and an LV ejection fraction <30% at presentation could already be defined.<sup>34</sup> As they become increasingly available, ventricular assist devices, such as the Berlin Heart for infants and children are used as a bridge to recovery.<sup>35</sup> Mechanical circulatory support represents an important and lifesaving therapeutic option in children with myocarditis with high weaning rates.<sup>34</sup> In summary, our study suggests that outcome is worse in children diagnosed with myocarditis and DCM phenotype. Children with myocarditis, but without DCM, have a high recovery rate. We suggest that heterozygous DCM causing LP/P variants are critical to predict outcome in myocarditis.

## Study Limitations

We systematically performed targeted panel next-generation sequencing (NGS) analysis of cardiac genes in pediatric myocarditis. The study would benefit from larger numbers in each subgroup which are difficult to obtain in the pediatric population; therefore, registries and multicenter studies are urgently needed.<sup>33,36</sup> As we were unable to recruit the parents of several families, segregation analysis of the identified variants was limited. This underestimates the detection of de novo variants and limits classification of LP/P variants according to ACMG. Genetic analysis by targeted NGS of CMP genes does not detect genetic defects modulating the innate or adaptive immune response.<sup>37</sup> However, a study using whole-exome sequencing did not identify genetic variation in

immune-modulatory genes.<sup>12</sup> Finally, we do not know whether and how long the DCM phenotype existed before admission in the MYC-DCM subgroup.

## Conclusions

We report heterozygous pathogenic genetic variants in biopsy-proven pediatric myocarditis. Myocarditis patients with DCM phenotype were likely to suffer from early-onset heart failure, with significant enrichment of rare genetic CMP variants, and poor outcome. These phenotype-specific and age group-specific findings will be useful for personalized management of these patients. Genetic evaluation in this subgroup of children, newly diagnosed with myocarditis and DCM phenotype is warranted.

## ARTICLE INFORMATION

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### Disclosures

None.

### Supplemental Materials

Expanded Materials and Methods  
Supplemental Tables I–VII  
Supplemental Figures I–III  
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