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ORIGINAL ARTICLE

Next-Generation Sequencing Reveals Novel Genetic Variants for Dilated Cardiomyopathy in Pediatric Chinese Patients

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Abstract

Dilated cardiomyopathy (DCM) is a myocardial disease characterized by bilateral or left ventricular cardiac dilation and systolic dysfunction that can lead to heart failure and sudden cardiac death in children. Many studies have focused on genetic variation in DCM-related genes in adult populations; however, the mutational landscape in pediatric DCM patients remains undetermined, especially in the Chinese population. We applied next-generation sequencing (NGS) technology to genetically analyze 46 pediatric DCM patients to reveal genotype–phenotype correlations. Our results indicated DCM-associated pathogenic mutations in 10 genes related to the structure or function of the sarcomere, desmosome, and cytoskeleton. We also identified 6 pathogenic mutations (5 novel) in the Titin (*TTN*) gene that resulted in truncated *TTN* variants in 6 (13%) out of 46 patients. Correlations between *TTN* mutations and clinical outcomes were assessed. Our data indicate that one-third of pediatric DCM cases are caused by genetic mutations. The role of *TTN* variants should not be underestimated in pediatric DCM and age-dependent pathogenic penetrance of these mutations should be considered for familial DCM cases. We argue that genetic testing of DCM cases is valuable for predicting disease severity, prognosis, and recurrence risk, and for screening first-degree relatives.

Keywords Dilated cardiomyopathy · Genetic testing · Pediatric patient · Titin · Mutation

Abbreviations

ACMG	American College of Medical Genetics and Genomics
ALT	Alanine transaminase
AST	Aspartate transaminase
CT	Computed tomography
DCM	Dilated cardiomyopathy
FLNC	Filamin C
HGMD	Human Gene Mutation Database
LVEDD	Left ventricular end-diastolic diameter
LVEF	Left ventricular ejection fraction
LVNC	Left ventricular noncompaction
MYH7	β-Myosin heavy chain
NEXN	Nexilin F-actin binding protein
NKX2-5	NK2 homeobox 5
NGS	Next generation sequencing
NT-proBNP	N-terminal pro-brain-natriuretic peptide
PRDM16	Positive regulatory domain16
RBM20	RNA-binding protein20
TNNI3	Troponin I3
TNNT2	Troponin T2
TTN	Titin
VCL	Vinculin
WES	Whole-exome sequencing

Introduction

Dilated cardiomyopathy (DCM) is the most common myocardial disease, characterized by left or bilateral cardiac dilatation and systolic dysfunction in the absence of any other comorbid condition [1]. It has an estimated prevalence of 1:2500 in the general adult population, but its prevalence among children is less at 1 in 170,000 in the USA and 1 in 140,000 in Australia [2–4]. DCM can be inherited and can lead to arrhythmias,

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heart failure, and sudden cardiac death (SCD) [5]. About 30–50% of DCM cases are of familial origin, and sporadic DCM is more commonly observed in pediatric patients [6]. Male individuals are three times more susceptible to DCM than females [7, 8]. DCM is a genetically heterogeneous disease [9, 10] that produces varied patient phenotypes resulting from the interaction of underlying genetic susceptibility and environmental factors.

More than 60 genes related to structural or functional components of the cytoskeleton, desmosome, nuclear lamina, sarcomere, and mitochondria, or calcium-binding are associated with DCM pathogenesis in a Mendelian autosomal dominant pattern [6, 8, 11, 12]. Mutations in these genes exhibit variable expressivity and penetrance in DCM [13]. Identifying disease-causing genetic variants in probands may help asymptomatic family members to assess their risk of developing cardiomyopathy [14]. Genetic diagnosis techniques, including next-generation sequencing (NGS) technology, have been extensively used correlate genetic mutation with phenotypic presentation in numerous human diseases [15]. Previous studies have primarily focused on the relationship between genetic mutation and clinical phenotype in adult DCM patients. Truncations in *Titin* (*TTN*) are the most common cause of DCM, occurring in 25% of familial and 18% of sporadic cases, with these variants are over-represented in the A-band region of the TTN protein [16].

Presently, the genetic landscape of pediatric DCM remains undetermined. Therefore, in this study, we assembled a cohort of 46 pediatric DCM patients. We present their clinical phenotypes and results from NGS analysis, including whole-exome sequencing and targeted gene panel analysis in combination with cardiomyopathy-related gene-filtering to identify underlying pathogenic mutations. We demonstrate association between genetic variation, phenotypic presentation, and clinical outcomes in pediatric DCM patients from Shandong province, China. Furthermore, this study provides a comprehensive landscape of genetic variation in pediatric DCM patients by applying stringent American College of Medical Genetics and Genomics (ACMG) criteria for classification. This can support genetic testing and counseling of patients. Moreover, our investigation highlights a clinical-pathological correlation between truncating mutations in *TTN*, a crucial component of muscle fibers, and clinical outcomes in pediatric DCM patients of Chinese genetic background.

Materials and Methods

Subjects and Clinical Evaluation

Patients diagnosed with DCM were recruited at the pediatric department of Shandong Provincial Hospital between 2014 and 2020. Patients with specific etiologies, such as congenital heart disease, myocarditis, rheumatic heart disease, systemic hypertension, cardiotoxicity, ischemic heart disease, metabolic and syndromic diseases, were excluded. The study cohort consisted of 46 patients ($n = 46$; mean age 6.5 months; range: 1–156). All parents of the study patients gave their informed consent for the study according to the Declaration of Helsinki. Echocardiographic measurements were indexed to age and body surface area, and corresponding *Z*-scores were derived whenever applicable. DCM was diagnosed when the *Z*-score of the left ventricular end-diastolic (LVEDD) and/or end-systolic (LVESD) volume was above 2 standard deviations (SD) of body surface area (based on Detroit data) [17], with left ventricular ejection fraction (LVEF) < 45% or fractional shortening < 20% in the absence of any comorbidities [18].

Probands ≤ 13 years old and available family members were evaluated according to their medical history, and by physical examination, 12-lead electrocardiography, and transthoracic echocardiography. We recorded clinical parameters, such as age at onset of DCM, sex, family history of DCM or SCD, LVEF, and LVEDD. Positive family history was defined as cardiomyopathy or SCD reported to a clinical geneticist at the time of evaluation. Whenever possible, a positive family history was confirmed by obtaining clinical records. We set the DCM recovery parameter as LVEF ≥ 55%. All patients were followed up in outpatient clinics or by telephone interview until December 31, 2020 and the mean follow-up time was 12.5 months (range: 1–84). The latest echocardiographic data and outcomes were recorded. For the survival analysis, event-free survival was calculated from the date of onset to the date of heart failure-related death.

Genetic Testing and Bioinformatics

Genomic DNA was extracted from peripheral blood samples using a QIAamp Blood Midi Kit (QIAGEN, Germany) according to the manufacturer's instructions. Two target panels were designed for NGS-based genetic analysis. Twenty-five patients were analyzed by Sinopath genetic technology (Beijing, China), which included a panel of 175 cardiac genes (Panel 1) (Online Resource Table 1) and 16 patients were analyzed by Novocardio genetic technology Co. Ltd (Beijing, China), which included a panel of 101 cardiomyopathy-related genes (panel 2) (Online Resource Table 2). The genomic DNA of the remaining five patients was analyzed by whole-exome sequencing (trio-WES) on the Illumina platform.

After filtering out low-quality reads, Burrows–Wheeler Aligner (BWA-MEM v0.7.12) was used to align the clean reads to the reference genome (UCSC Genome Browser hg19) for sorting and duplicate marking. Insertion and/or

deletion (InDel) sequence determination and base quality score calibration were carried out by local realignment using Genome Analysis Toolkit software (GATK v3.2) [19]. Single-nucleotide polymorphisms and InDel calling were performed by GATK's Haplotype caller [20]. All determined variants were annotated by ANNOVAR and searched for in multiple databases, including 1000 genome, ESP6500, dbSNP, EXAC, and HGMD (Human Gene Mutation Database). Variant effects were predicted using SIFT, PolyPhen-2(PP2), MutationTaster (MT), and GERP++.

The pathogenicity of all variants was assessed in accordance with ACMG guidelines [21]. Sanger sequencing was then performed to confirm the presence of pathogenic or likely pathogenic variants and their parental origins.

Statistical Analysis

Statistical analysis was performed with SPSS (version 26.0) software. All data are expressed as the mean ± SD or, for non-parametric values, as the median and lower and upper quartiles. The difference in continuous variables was assessed by Student's *t*-test and the Mann–Whitney *U* test was used when the distributions were asymmetrical. Characteristics of different groups, such as patients with or without disease-causing mutations, were compared using the chi-square test for categorical variables if appropriate; otherwise, Fisher's exact test was used. The Kaplan–Meier method was used to calculate survival, and the log-rank test was used to compare survival curves between different patient groups. All statistical tests were two-sided, and *P* values < 0.05 were statistically significant.

Results

Clinical Characteristics

The clinical characteristics of 46 pediatric patients with DCM onset (26 female and 20 male) are presented in Tables 1 and 2. Almost all patients presented with a common respiratory syndrome-like shortness of breath and cough. Notably, heart rhythm disturbances [atrial tachycardia (1 patient), premature ventricular contractions with low voltage (2 patients), transient junctional rhythm (1 patient), premature ventricular contractions (7 patients), left anterior fascicular blocks (2 patients), left bundle branch block (1 patient), and atrial premature beats (3 patients)] were observed in 17 patients. It is important to note that 67.4% of patients (31/46) manifested the disease before 1 year of age with a median age at diagnosis of 6.5 months, and 84.8% (39/46) of patients were diagnosed before the child's third birthday. At presentation, the mean LVEF was 28.5%, and the mean LVEDD *Z*-score was 7.02. The mean serum N-terminal pro-brain natriuretic peptide (NT-proBNP) level was 14,406 pg/ml, which is much higher than the reference range (< 125 pg/ml) (Table 3). At the median follow-up of 12.5 months, 54.3% of patients (25/46) had recovered from DCM and their LVEF was within the normal range. Ten out of 46 patients died because of severe DCM and the average time of death was 1–36 months (median 7 months) after the initial diagnosis. Importantly, 90% of deaths occurred within the first year after diagnosis, a critical time period reflected by the significant morbidity and mortality. Genetic analysis of DCM showed high heterogeneity in these patients with only three patients having a familial history of DCM. No significant differences were observed between the sexes for LVEF and LVEDD *Z*-scores, age of onset, serum NT-proBNP level at diagnosis, follow-up time, or death, or recovery outcome (all $P > 0.05$) (Table 2).

Table 1 Age distribution of pediatric patients with DCM onset

Age group, years	*N*		Death during follow-up (*n*)
	Females	Males	
< 1	17	14	5
1–3	5	3	2
3–6	1	0	0
6–10	1	0	1
10–13	2	3	2
Total	26	20	10

Genetic Characteristics of the Genotype-Positive Group

Based on the sequencing results, the cohort was divided into two groups: a genotype-positive group (16/46) and a genotype-negative group (30/46), as shown in Table 3. Considering the stringent selection criteria and reclassification of DCM according to the ACMG guidelines, the genetic analysis indicated that the 16 genotype-positive patients could be classified as either 'pathogenic' or 'likely pathogenic' mutant carriers. All carriers had only one pathogenic or likely pathogenic mutation in DCM-associated genes (Fig. 1). We identified 10 genes with disease-causing heterozygous mutations. These were genes with integral sarcomere functions: Titin (*TTN*) [(OMIM*604145), ($n = 6$, 37.5%)], Myosin heavy chain 7 (*MYH7*) [(OMIM*613426) ($n = 1$, 6.25%)], Troponin T2 (*TNNT2*) [(OMIM *601494) ($n = 1$, 6.25%)], Nexilin (*NEXN*) [(OMIM*613122) ($n = 1$, 6.25%)], Troponin I3 (*TNNI3*) [(OMIM*613286) ($n = 1$, 6.25%)]; cytoskeletal structure-related genes: Filamin-C (*FLNC*) [(OMIM*617047) ($n = 1$, 6.25%)], Vinculin (*VCL*) [(OMIM*611407) ($n = 1$, 6.25%)]; and other genes: RNA-binding motif Protein 20 (*RBM20*) [(OMIM*613172) ($n = 2$, 12.5%)], NK2 homeobox 5 (*NKX2-5*) [(OMIM:108900)

Table 2 Comparison of pediatric DCM patient clinical characteristics between females and males

	All patients, $n=46$		*P*-value
	Females, $n=26$	Males, $n=20$	
Age, months (range)	7 (1–144)	6 (1–156)	0.657
LVEF % (range)	30 (20–42)	27.5 (17–35)	0.355
LVEDD *Z*-score (range)	7.46 (2.36–13.65)	6.75 (3.01–12.87)	0.807
NT-proBNP pg/ml (range)	15466 (84.55–35000)	6485 (960–35000)	0.594
Died during study, *n* (%)	6 (23.1)	4 (20)	0.059
Recovered during study, *n* (%)	15 (60)	10 (50)	0.608

LVEF left ventricular ejection fraction, *LVEDD* left ventricular end-diastolic diameter (*Z*-score, normal reference range between − 2 standard deviations [SD] and + 2 SD); *NT-proBNP* N-terminal fragment of pro-b-natriuretic peptide

Table 3 Clinical characteristics of genotype-positive and genotype-negative groups

Clinical characteristics	All ($n=46$)	Genotype positive ($n=16$)	Genotype negative ($n=30$)	*P*-value
Sex				0.202
Male, *n* (%)	20 (43.5)	9 (56,25)	11 (36.7)	
Female, *n* (%)	26 (56.5)	7 (43.75)	19 (63.3)	
Age at onset				
Months (range)	6.5 (1–156)	9.5 (1–156)	6 (1.5–108)	0.23
< 12 months, *n* (%)	31 (67.4)	9 (56.2)	22 (73.3)	
12–156 months, *n* (%)	15 (32.6)	7 (43.8)	8 (26.7)	
LVEF % (range)	28.5 (17–42)	28.5 (20–33)	28.5 (17–42)	0.935
LVEDD *Z*-score (range)	7.02 (2.36–13.65)	6.4 (3.6–13.65)	7.08 (2.36–12.87)	0.42
NT-proBNP pg/ml (range)	14,406 (54.5–35,000)	6276 (960.4–35,000)	16,305 (84.5–35,000)	0.115
Follow-up (months) (range)	12.5 (1–84)	12 (1–84)	12.5 (1–69)	0.972
Died during study, *n* (%)	10 (21.7)	6 (37.5)	4 (13.3)	0.06
Arrhythmia *n* (%)	17 (36.9)	10 (62.5)	7 (23.3)	0.01
Recovered during study, *n* (%)	25 (54.3)	8 (50)	17 (56.7)	0.665

LVEF Left ventricular ejection fraction, *LVEDD* left ventricular end-diastolic diameter (*Z*-score, normal reference range between − 2 standard deviations [SD] and + 2 SD), *NT-proBNP* N-terminal pro-brain natriuretic peptide

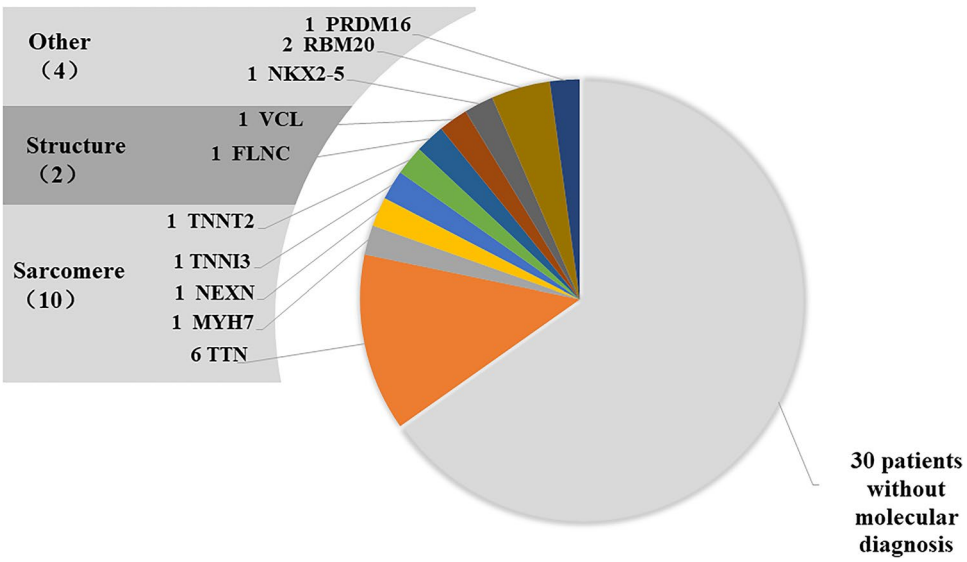

Fig. 1 Distribution of pathogenic or likely pathogenic variants in the DCM cohort. Distribution of pediatric DCM patients based on mutations in genes related to either sarcomere, cytoskeletal structure, or other cellular functions. Thirty 30 out of 46 patients had no underlying genetic mutation related to DCM pathogenesis. The majority of mutation-positive patients exhibited pathogenic or likely pathogenic mutations in sarcomeric genes

($n=1$, 6.25%)], and PR domain containing 16 (*PRDM16*) [(OMIM*615373) ($n=1$, 6.25%)]. Table 4 summarizes the main clinical features and the details of identified variants in DCM patients. Three patients had familial mutations in *TTN*, *MYH7*, and *NEXN* genes, and five de novo variants were identified in *NKX2-5*, *TNNI3*, *PRDM16,* and *RBM20* ($n=2$). Among the 16 mutations identified (5 missense, 5 nonsense, 4 frameshift, and 2 splice site), 2 were identified in a pair of monozygotic twin patients, and 10 (62.5%) were novel.

In total, we identified six (five novel) *TTN* truncation variants, including three nonsense, one frameshift, and two splice-site variants, in 13% of patients (Table 4). Furthermore, we mapped the identified variants to protein domains of *TTN* (Online Resource Fig. 1a). Consistent with previous reports, this showed that four variants were in the I-band region, and the remaining two were in the A-band and M-band regions, respectively.

Genotype and Clinical Phenotype Analyses

The severity of patient phenotype at presentation was assessed by LVEDD *Z*-score, LVEF, and serum NT-proBNP levels. There were no significant differences in distribution based on sex ($P=0.202$), age ($P=0.23$), LVEF ($P=0.935$), LVEDD *Z*-score ($P=0.42$), or serum NT-proBNP levels ($P=0.115$) between genotype-positive and genotype-negative groups. More cardiac arrhythmia was observed in genotype-positive patients ($P=0.01$). The genotype-positive group probands exhibited lower phenotypic severity (echocardiographic parameters) than genotype-negative group probands, with the same LVEF of 28.5% and LVEDD *Z*-score of 6.4 versus 7.08, respectively. We also observed a trend that the onset age (median 9.5 months) in the genotype-positive group was slightly higher than that in the genotype-negative group (median 6 months). Nine genotype-positive patients developed DCM before 12 months of age, and the age distribution was between 1 month and 13 years.

Despite better echocardiographic parameters and seemingly better phenotypes at presentation in genotype-positive patients, there was no significant difference in outcome (the number of deceased patients, $P=0.145$ and LVEF recovery patients, $P=0.665$) between the two groups during the 12.5 months (median) follow-up time ($P=0.972$). Heart failure-related death occurred in 37.5% (6/16) of genotype-positive patients compared with only 13% (4/30) of genotype-negative patients. No significant differences in survival ($P=0.093$) were documented. Cardiac-related death occurred in patients with truncating mutations in *TTN* (c.50065C>T, c.98421_98422insGG, c.37454–2A>T), *NKX2-5* (c.242delA), *TNNT2* (c.422G>A), and *TNNI3* (c.544G>A). Notably, five out of six disease-causing mutations belong to sarcomeric genes (*TT* $n=3$, *MYH7*, *TNNT2*, and *TNNI3*).

A significant proportion of the patients exhibited marked improvement and better prognostic outcomes in response to heart failure treatment. In both groups, almost 50% of pediatric patients recovered (LVEF above 50%).

The clinical and genetic characteristics of four probands with four truncating *TTN* mutations and of twin brothers with *RBM20* mutations are summarized in Table 4 and in Online Resource Figs. 1–3. We identified the functional domains of the *TTN* protein and showed that the DCM-linked pathogenic or likely pathogenic mutations reside mostly in the I-band domain; other mutations were scattered in the A-band and M-band. The TTN protein sequence is highly conserved among different vertebrates and the residues corresponding to mutation sites are shown in the various *TTN* proteins in Online Resource Fig. 1. The pedigree tree and Sanger sequencing which confirmed the variants are shown in Online Resource Fig. 2.

Discussion

DCM is a common pediatric heart disease that can lead to poor clinical outcomes and heart transplantation [22, 23]. In this cohort, with a mean LVEF of 28.5% and a mean LVEDD *Z*-score of 7.02, DCM presented as a severe disease and 67.4% of patients (31/46) manifested DCM before 1 year of age. DCM affects men more commonly than women. In a large heart disease cohort, male sex was an independent predictor of mortality, and women with heart failure had better transplant-free survival compared with men [24, 25]. Another study also showed better prognosis for women with DCM [25]. However, in the present study, no significant sex difference was observed for LVEF, LVEDD *Z*-scores, age at onset, serum NT-proBNP level, either at diagnosis or follow-up, or for the outcome of death, or recovery (all $P>0.05$). This may be because our patient sample size was relatively small, although ethnic differences should also be considered.

The prognosis of pediatric DCM is usually bad with a high mortality rate [5]. In this cohort, although 21.7% (10/46) of patients died after medical therapy without heart transplantation, half of the patients recovered reflecting major advances in medical technology. A limitation of our and similar studies is that patients who agree to participate may not completely reflect the recovery ratio in the general population. However, this positive prognosis is worthy of further study and we believe that a well-balanced, large population-based study is warranted.

Genetic factors play an important role in DCM pathogenesis. However, genetic and prognostic understanding are still a challenge for DCM therapy. Hence, we sought to reveal genetic variations that are prevalent in pediatric patients with DCM. Among our 46 pediatric patients with DCM, 16 (34.8%) carried at least one pathogenic or likely

Table 4 Clinical and genetic characteristics of genotype-positive group patients

Patient #	Sex	Age at onset	Family history	Time to death after diagnosis	Current age	LVEF recovery (above 55%)	Arrhythmia	Gene	Variant (DNA protein)	Method	Previous reports of the variant
P1	M	12 months	No	–	3 years	Yes	Sinus rhythm	*TTN*	c.18230–1G>A splicing	Panel 2	No
P2	F	12 years	No	–	14 years	Yes	Premature ventricular contractions with low voltage	*TTN*	c.43298T>G p.Leu14433*	Panel 2	No
P3	F	5 months	No	–	1.9 years	Yes	Transient junctional rhythm	*FLNC*	c.4249_4250del p.Y1417*fs	Panel 2	No
P4	M	12 years	No	3 months	–	No	Left anterior fascicular block	*TTN*	c.50065C>T p.Arg16689*	Panel 2	[42]
P5	M	10 months	No	4 months	–	No	Sinus rhythm	*NKX2-5*	c.242delA p.K81Sfs*95	Panel 1	No
P6	M	13 years	No	–	21 years	Yes	Low voltage, premature ventricular contractions	*RBM20*	c.2746G>A p.E916K	WES	[43]
P7	M	13 years	No	–	21 years	Yes	Low voltage	*RBM20*	c.2746G>A p.E916K	WES	[43]
P8	F	10 years	No	36 months	–	No	Premature ventricular contractions	*TTN*	c.98421_98422insGG p.I32808Pfs*7	Panel 1	No
P9	F	8 months	No	–	4 years	Yes	Premature ventricular contractions	*PRDM16*	c.764C>G p.S255*	Panel 1	No
P10	M	1 month	Brother, DCM	–	7 months	No	Atrial premature beats	*MYH7*	c.2713T>C p.C905R	WES	[44]
P11	F	9 months	No	–	4 years	No	Premature ventricular contractions	*VCL*	c.142C>T p.Q48*	Panel 1	No
P12	F	1.5 months	No	1 month	–	No	Sinus rhythm	*TTN*	c.37454–2A>T splicing	Panel 1	No
P13	F	3 months	Mother and grandfather, DCM	–	4 years	Yes	Atrial tachycardia	*TTN*	c.105541A>T p.K35181*	WES	No
P14	M	3 months	Brother, heart enlarged and death	–	6.5 years	Yes	Atrial premature beats	*NEXN*	c.1139_1142del p.E380Dfs*2	Panel 1	No
P15	M	18 months	No	1 month	–	No	Sinus rhythm	*TNNT2*	c.422G>A p.R141Q	Panel 1	[45, 46]
P16	M	3.5 months	No	2 months	–	No	Premature ventricular contractions	*TNNI3*	c.544G>A p.E182K	WES	[47–49]

Patient #	Mutation type	Inheritance	GnomAD	SIFT and score	MT and score	PP2 and score	GERP++ and score	REVEL	ACMG variant classification
P1	Splicing	Unknown	–	–	D (1)	–	C (5.95)	–	Likely pathogenic PVS1; PM2
P2	Nonsense	Maternal	–	–	A (1)	–	C (5.56)	–	Likely pathogenic PVS1; PM2

Table 4 (continued)

Patient #	Mutation type	Inheritance	GnomAD	SIFT and score	MT and score	PP2 and score	GERP++ and score	REVEL	ACMG variant classification
P3	Frameshift	Paternal	–	–	–	–	–	–	Likely pathogenic PVS1; PM2
P4	Nonsense	Maternal	–	–	A (1)	–	C (5.08)	–	Pathogenic PVS1; PM3_Strong; PM2
P5	Frameshift	De novo	–	–	–	–	–	–	Likely pathogenic PVS1; PM2
P6	Missense	De novo	–	D (0.024)	N (0.849)	P (0.874)	C (4.71)	B (0.244)	Likely pathogenic PS4; PM2; BP4
P7	Missense	De novo	–	D (0.024)	N (0.849)	P (0.874)	C (4.71)	B (0.244)	Likely pathogenic PS4; PM2; BP4
P8	Frameshift	Paternal	–	–	–	–	–	–	Likely pathogenic PVS1; PM2
P9	Nonsense	De novo	–	–	A (1)	–	C (3.39)	–	Likely pathogenic PVS1; PM2
P10	Missense	Maternal	–	D (0)	D (1)	P (0.998)	C (5.55)	D (0.892)	Pathogenic PM3_Strong; PM1, PM2, PM5, PP3
P11	Nonsense	Maternal	–	–	A (1)	–	C (4.41)	–	Likely pathogenic PVS1; PM2
P12	Splicing	Unknown	–	–	D (1)	–	C (4.34)	–	Likely pathogenic PVS1; PM2
P13	Nonsense	Maternal	–	–	A (1)	–	C (4.78)	–	Likely pathogenic PVS1; PM2
P14	Frameshift	Paternal	–	–	–	–	–	–	Likely pathogenic PVS1; PM2
P15	Missense	Maternal	0.000008	D (0)	D (1)	P (0.999)	C (4.28)	D (0.501)	Likely pathogenic PS1; PM1; PP3
P16	Missense	De novo	–	T (0.364)	D (1)	B (0.005)	C (4.72)	B (0.319)	Likely pathogenic PM3_Strong, PM2, BP4

ACMG American College of Medical Genetics and Genomics, *CADD* combined annotation-dependent depletion scores, *DCM* dilated cardiomyopathy, *PM* pathogenic moderate, *PP* pathogenic supporting; *PS* pathogenic strong, *BP* supporting benign, *PVS* pathogenic very strong, *SIFT* Sorting Intolerant from Tolerant tool, *WES* whole-exome sequencing, *panel 1* Sinopath genetic technology including 175 cardiac genes, *panel 2* Novocardio genetic technology Co. Ltd (Beijing, China), including 101 cardiomyopathy-related genes. P6 and P7 were twin brothers. SIFT Sorts intolerant from tolerant (*D* damaging, *T* tolerant), *PP2* polymorphism phenotyping v2 (*D* damaging, *P* possible damaging, *B* benign), MT mutation taster (*D* disease causing, *A* disease causing automatic, *N* polymorphism, *P* polymorphism_automatic), GERP++ genomic evolutionary rate profiling (*C* conserved, *N* non-conserved), Revel (*D* damaging, *B* benign)

pathogenic mutation in a disease-causing gene. Consistent with previous studies, mutations were most frequently detected in sarcomere genes in all 46 DCM patients [26]. The prevalence of pathogenic mutations seemed to be similar to those in recently published cohorts [16, 27]. A recent multicenter study in North America found 35% of familial DCM and 9% of idiopathic DCM patients carried pathogenic or likely pathogenic mutants; however, only two truncating *TTN* mutations were identified [28]. These high mutation rates cannot be ignored and further confirmed the value of genetic testing in DCM. There was no difference in clinical presentation or prognosis between genotype-positive and genotype-negative groups, except for arrhythmia.

Mutations are frequently identified in child-onset DCM, especially in sarcomeric genes. Importantly, we found *TTN*-related mutations in 10 out of 16 genotype-positive patients. The truncated *TTN* carrier rate was 0.6–1.2% in the USA, and the odds ratio for DCM in patients of European ancestry was 10.8–18.7% [29]. The truncated *TTN* carrier rate in the general Chinese population is unknown. Truncating *TTN* mutations account for 12–27% of all adult DCM cases, indicating the importance of diagnostic sequencing [30–34]. However, truncating *TTN* mutations have been rarely identified in pediatric patients [35–37] and recent studies in pediatric DCM have shown similar results [14]. Notably, genetic analysis of a 66-patient cohort of severe childhood cardiomyopathy, including 37 DCM cases, did not identify any truncating *TTN* mutations [32]. Likewise, another study involving 30 Chinese pediatric patients with sporadic DCM pathology did not find any pathogenic truncating *TTN* mutations [33]. However, in another study only one pathogenic truncating *TTN* variant was identified in a 16-year-old boy among 36 pediatric DCM patients [34]. A study of 70 pediatric probands, including 56 DCM patients who underwent genetic evaluation, showed that 16 carried pathogenic mutations but only three *TTN* mutations were identified [36]. Our results are clearly different from these findings; we identified six different truncating *TTN* variants in 6 (13%) of 46 pediatric patients.

Arrhythmias were more common in the genotype-positive group (10/16) ($P = 0.01$). Consistently, many studies have identified early and life-threatening arrhythmias in DCM associated with gene mutations, especially truncating *TTN* mutations and *LMNA* mutations, although the mechanism for this association remains incompletely understood [38, 39]. The types of arrhythmia also vary, including atrial or ventricular arrhythmia. Endomyocardial interstitial fibrosis may be a factor of arrhythmia in *TTN*-DCM [39] and the correlation between arrhythmia and DCM genotype should be investigated further. The prognosis of DCM patients with truncating *TTN* mutations is different and inconsistent in adults with moderate to severe outcomes. Notably, DCM patients with *TTN* mutations can have a good response to treatment [35]. A cohort of 70 patients with end-stage DCM showed recovery after left ventricle assist device implantation [36]. Another study showed that *TTN* mutation-positive patients frequently present severe cardiomyopathy and a worse 5-year prognosis [4].

We also observed different clinical outcomes in *TTN* genotype-positive patients. Three patients with *TTN* mutations (c.18230–1G>A, c.43298T>G, and c.105541A>T) recovered after medical treatment with no further symptoms, while three other patients harboring *TTN* mutations (c.50065C>T, c.98421_98422insGG, and c.37454-2A>T) showed no response to treatment and died from heart failure, indicating clinical heterogeneity. These conflicting prognoses indicate that apart from the identified genetic factors, post-transcriptional, environmental, hormonal, and other factors may also modify the rate of disease progression. Therefore, it might be difficult to predict clinical outcomes in pediatric DCM cases based on truncating *TTN* mutations alone.

The penetrance of truncating *TTN* mutations can reach up to 100% by the age of 70 years [40], which leads to discordant segregation with phenotype, and the same variant has also been detected in unaffected relatives. In some pedigrees, especially for familial DCM, clinical follow-up with aging should be performed for unaffected relatives. The age at onset of girl **P13** (3 months) with a truncating *TTN* mutation (c.105541A>T, p.K35181X) was obviously different from that of her mother (23 years) and her grandfather (36 years), indicating the possible involvement of other factors.

We also identified a de novo pathogenic *RBM20* variant (p.E916K) in twin patients (**P6** and **P7**) with pediatric DCM. *RBM20* mutations have been associated with cardiomyopathy [41]. Notably, the *RBM20* variant c.2746G>A (p.E916K) identified in this study was located in exon 11, a known hot spot for cardiomyopathy-associated mutations (Online Resource Fig. 2). However, the mechanistic relationship of *RBM20* mutations with DCM onset is still unclear.

A de novo mutation in *PRDM16* in an 8-month-old girl (**P9**) was associated with pediatric DCM. Previously identified mutations in this gene are mostly missense mutations; however, a nonsense mutation leading to functional loss of PRDM16 was detected in this case. The role of *PRDM16* mutations could be very important, warranting further studies to investigate their pathogenicity.

This study suffers from the following limitations: (1) it was a single-center, retrospective, and a small cohort study; (2) follow-up time was not long enough to estimate long-term outcomes; (3) clinical assessment was highly recommended for family members of the probands, but only a minority of the relatives were willing to participate in this evaluation; therefore, gene mutation carriers in a family could be underestimated in our cohort; (4) bioinformatic prediction can give useful information about the

pathogenicity of mutants associated with DCM; however, it cannot reveal the real pathobiology of mutations in cardiac myocytes; (5) this study lacked analysis of endomyocardial biopsies.

In conclusion, DCM is a genetically heterogeneous disease in children and adults. Using genetic testing (NGS analysis), we detected that more than one-third of DCM cases were caused by mutations in genes related to the structure or function of the sarcomere, desmosome, or cytoskeleton. We also assessed genotype–phenotype correlations in pediatric DCM patients. We discovered six truncating *TTN* mutations (five of which were novel) that correlated with severe disease phenotypes. Furthermore, we identified 16 mutations in 10 genes in 16 patients that were likely to be associated with DCM pathogenesis. Most pediatric patients were diagnosed with DCM before 1 year of age. Also, most deaths occurred within the first year of life after diagnosis. Death occurred in patients harboring mutations in *TTN* (3 patients), *NKX2-5* (1 patient), and *TNNT2* (1 patient). Hence, this study advances the genetic understanding of pediatric DCM and highlights certain mutations with severe clinical courses. Further studies are needed to define the mechanisms by which pathogenic *TTN* variants affect outcomes in pediatric and adult patients with DCM. DCM may have a molecular cause that can be identified through genetic testing.

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Author Contributions All authors contributed to study conception and design. YW and BH collected patient data and prepared the manuscript. YF, XY, JL, and JW contributed to the clinical evaluation of patients and revision of the manuscript. YY, HY, LZ, and JZ analyzed and interpreted the genetic data and surveyed the literature relevant to the mutations. All authors reviewed the results and approved the final version of the manuscript.

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Data Availability The data and materials are available upon request.

Code Availability N/A.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Consent to Participate N/A.

Consent for Publication All authors and study participants declare their unconditional consent toward publication of this manuscript and its associated data in a peer-reviewed journal.

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