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Cardiovascular genetics – focus on paediatric cardiomyopathy

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Cardiovascular genetics – focus on paediatric cardiomyopathy

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Editorial: Cardiovascular genetics—focus on paediatric cardiomyopathy

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cardiomyopathy, hypertrophic cardiomyopathy, dilated cardiomyopathy, arrthythmogenic right ventricular cardiomyopathy, restrictive cardiomyopathy

Editorial on the Research Topic
Cardiovascular genetics—focus on paediatric cardiomyopathy

Cardiomyopathies (CMPs) are a large group of disorders characterized by structural, functional, and electrical abnormalities of the heart muscle (1). These conditions are rare in childhood but associated with increased mortality and morbidity (1). Over the last few years, data on the genetic basis, natural history, risk stratification, and treatment of childhood CMPs have emerged.

In this Research Topic, experts in the field explored this poorly characterised area with comprehensive and state of the art reviews, original articles, and challenging case reports, dealing with molecular basis, role of genetic testing, clinical manifestations, outcome, and management of childhood CMPs.

This Research Topic collected 14 articles, and we are pleased to introduce them to readers of *Frontiers in Pediatrics*.

Cardiomyopathies

Childhood CMPs include a large spectrum of diseases, with outcome mainly depending on phenotype, aetiology, and clinical characteristics (2). CMPs are generally categorized into 5 main phenotypes, namely hypertrophic (HCM), dilated (DCM), restrictive (RCM), arrhythmogenic (ACM), and left ventricular non-compaction (LVNC) cardiomyopathy (3). In this regard, Rath et al. provided an overview of childhood CMPs, with a focus on epidemiology, natural history, and outcomes according to clinical phenotype and aetiology.

Recently, it has been observed that morphological, functional, and electrical abnormalities of the heart muscle may be part of the spectrum of Marfan syndrome and heritable thoracic aortic disease (4), contributing to morbidity and mortality of these patients. In their review, Laura Muiño-Mosquera et al. reported data on preclinical studies (i.e., mouse models of Marfan syndrome) and described the clinical presentation of cardiovascular involvement of children and adults with genetic aortic diseases.

Hypertrophic cardiomyopathy

HCM is a myocardial disease characterized by increased left ventricular wall thickness not solely explained by abnormal loading conditions (5). It represents the second most common

cause of CMP presenting in childhood. The aetiology of childhood HCM is more heterogenous than seen in the adult population (6), and includes genetic syndromes (e.g., RASopathies) (7), inborn errors of metabolism (8), and neuromuscular disorders (9).

Among the different causes of HCM in childhood, mutations in sarcomeric genes account for the large majority of cases. Norrish et al. provided a comprehensive review of sarcomeric HCM in children, highlighting the variability in disease expression and unanswered questions. In contrast, Monda et al. presented a detailed review of the non-sarcomeric causes of HCM in children, focusing on their pathophysiology, clinical features, diagnosis, and etiological therapy.

In the large spectrum of HCM aetiologies, inborn errors of metabolism represent an extremely rare cause. For example, methylmalonic acidaemia is an inherited disease caused by mutations in different genes, which result in impairment of methylmalonyl-CoA mutase or impaired intracellular cobalamin transport and processing (10). Large studies describing the cardiac involvement of patients with methylmalonic acidaemia are lacking. Thus, Liu et al. screened 99 patients with methylmalonic acidaemia using electrocardiography and echocardiography. In their original article, the authors described the prevalence of congenital heart disease, cardiomyopathy, and pulmonary hypertension, and detailed electrocardiographic ed echocardiographic characteristics of patients with this rare life-threatening disease.

Exome sequencing demonstrated a high diagnostic yield in achieving a genetic diagnosis of rare disorders (11), and its role in the diagnosis of rare CMPs is expanding (12). In this Research Topic, two well-characterized case reports of patients with rare mitochondrial disorders associated with HCM diagnosed using exome sequencing are described. Wang et al. described two cases of Sengers syndrome, a rare autosomal recessive disorder due to mutations in acylglycerol kinase (AGK) gene. Both cases exhibited the classic clinical presentation of Sengers syndrome, including HCM, bilateral cataract, myopathy, and lactic acidosis. Furthermore, they provided a comprehensive literature systematic review of previously published cases and discussed the importance of exome sequencing for the diagnosis of rare genetic diseases. Moreover, Wang et al. described the cases of a 14-year-old boy with HCM, exercise intolerance, ptosis, and lactic acidosis, and his 9-year-old brother with similar clinical feature. Using exome sequencing, these two patients were diagnosed with early onset combined oxidative phosphorylation deficiency 33 (COXPD33), a rare mitochondrial disease caused by a homozygous mutation in the C1QBP gene. Finally, Wang et al. in their mini-review, summarized the physiological function of complement C1q binding protein (C1QBP) and its mutation-associated mitochondrial CMP.

Dilated cardiomyopathy

Dilated cardiomyopathy is a myocardial disease characterized by left ventricular dilation and dysfunction not solely explained by congenital heart disease, valve disease, or coronary artery disease (13). DCM is the most common cause of childhood CMP and is associated with a high risk for mortality (14). Several varieties of causation have been described for DCM and are generally classified as genetic and non-genetic causes (1). Among genetic causes of childhood DCM, a rare cause is represented by neuromuscular disorders (NMDs). NMDs may be inherited in autosomal dominant or recessive, or X-linked recessive patterns. Emery-Dreifuss muscular dystrophy is dominated by skeletal muscle weakness and cardiac involvement. However, cardiac abnormalities rarely appear in paediatric age. Kovalchuk et al. described five patients with different forms of Emery-Dreifuss muscular dystrophy caused by mutations in EMD or LMNA genes, presented with early-onset of cardiac abnormalities and no prominent skeletal muscle phenotype.

The pathophysiology of DCM is complex and not fully elucidated. Recently, it has been reported that long non-coding RNAs (lncRNAs) play a crucial role in regulating disease presentation and severity of several cardiovascular diseases (15). Cai et al. evaluated the expression profile of lncRNAs in children with DCM and explored their possible function, providing new relevant perspective for future research.

Arrhythmogenic cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a myocardial disease characterized by ventricular arrhythmias and ventricular abnormalities, mainly manifesting as right ventricular dilation and/or dysfunction, caused by progressive fibrofatty replacement of the myocardium (16). ARVC very rarely manifests in childhood, and when it occurs is generally associated with worse outcome (17). Due to its rarity, several gaps in knowledge still exist. In their clinically focused review, Te Riele et al. describes the spectrum of childhood ARVC, the genetic architecture of the disease, and current diagnostic and therapeutic strategies.

Restrictive cardiomyopathy

RCM is a myocardial disease characterized by abnormal diastolic function with restrictive physiology and normal ventricular diameter, wall thickness and systolic function (1). While two thirds of children have pure RCM phenotype, the remaining part has a mixed RCM-HCM phenotype (18). Children with RCM present the worst outcomes among any paediatric CMP group. In their review, Ditaranto et al. summarized the causes of childhood RCM, their pathophysiology, clinical presentation, and management.

Left ventricular non-compaction cardiomyopathy

LVNC is a myocardial disease characterized by prominent left ventricular trabeculae and deep intertrabecular recesses (1). LVNC is a very heterogeneous condition ranging from asymptomatic cases to severely affected individuals with need for transplantation or at risk for sudden cardiac death. Schultze-Berndt et al. described the genotype-phenotype correlation and clinical outcome of a cohort of 149 patients (both paediatric and adult) with LVNC.

They found that reduced left ventricular systolic function was the main independent predictor for adverse events.

Finally, next to articles purely focused on CMPs, Sarquella-Brugada et al. provide a comprehensive description of genotype-phenotype correlation of all rare variants in TRDN leading to malignant arrhythmias in paediatric patients.

The editors hope that readers of this Research Topic will find it of interest.

Author contributions

EM, JPK and GL contributed equally to the conception and drafting of the editorial. All authors contributed to the article and approved the submitted version.

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.

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ORIGINAL RESEARCH
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Early Onset of Combined Oxidative Phosphorylation Deficiency in Two Chinese Brothers Caused by a Homozygous (Leu275Phe) Mutation in the *C1QBP* Gene

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Mitochondrial diseases constitute a group of heterogeneous hereditary diseases caused by impairments in mitochondrial oxidative phosphorylation and abnormal cellular energy metabolism. C1QBP plays an important role in mitochondrial homeostasis. In this study, clinical, laboratory examinations, 12-lead electrocardiographic, ultrasonic cardiogram, and magnetic resonance imaging data were collected from four members of a Chinese family. Whole exome were amplified and sequenced for the proband. The structure of protein encoded by the mutation was predicted using multiple software programs. The proband was a 14-year old boy with myocardial hypertrophy, exercise intolerance, ptosis, and increased lactate. His 9-year old brother exhibited similar clinical manifestations while the phenomenon of ptosis was not as noticeable as the proband. The onset of this disease was in infancy in both cases. They were born after uneventful pregnancies of five generation blood relative Chinese parents. A homozygous mutation (Leu275Phe) in the *C1QBP* gene was identified in both brothers in an autosomal recessive inherited pattern. Their parents were heterozygous mutation carriers without clinical manifestations. We demonstrated that a homozygous C1QBP- P.Leu275Phe mutation in an autosomal recessive inherited mode of inheritance caused early onset combined oxidative phosphorylation deficiency 33 (COXPD 33) (OMIM:617713) in two brothers from a Chinese family.

Keywords: C1QPB, Leu275Phe, combined oxidative phosphorylation deficiency, mitochondrial cardiomyopathies, hypertrophic cardiomyopathy

INTRODUCTION

Mitochondrial disease is a type of inherited metabolic disorder caused by defects in mitochondrial metabolic enzymes that result in disorders pertaining to adenosine triphosphate (ATP) synthesis and in insufficient energy sources. Mitochondrial dysfunction primarily affects organs with high-energy requirements, such as the heart, brain, and muscles (1, 2). To-this-date, ∼300

genes associated with mitochondrial disease have been identified (3). Mutations can directly affect oxidative phosphorylation (OXPHOS) subunits or indirectly impair OXPHOS activity by disrupting mitochondrial homeostasis (4). Primary deficiencies of the OXPHOS system have direct impacts on mitochondrial function and result in several disease phenotypes, such as mitochondrial cardiomyopathies, mitochondrial encephalomyopathies, and mitochondrial myopathies (5).

Complement component 1 Q subcomponent-binding protein (C1QBP), also known as p32, is an evolutionary conserved and ubiquitously expressed multifunctional protein (6). Additionally, it is a predominant mitochondrial matrix protein involved in inflammation and infection processes, mitochondrial ribosome biogenesis, regulation of apoptosis and nuclear transcription, and pre-messenger ribonucleic acid (mRNA) splicing (7–10). C1QBP dysfunction could lead to a reduction of OXPHOS enzymes and mitochondrial energy metabolism disorders that may be attributed to a severely impaired mitochondrial protein synthetic process (11). Up to now, only six cases were reported with the mutations in the C1QBP gene in an autosomal recessive pattern. Feichtinger et al. (12) reported four individuals with biallelic mutations in C1QBP. The biallelic mutation of C1QBP caused a combined oxidative phosphorylation deficiency 33 (COXPD 33) (OMIM:617713). COXPD 33 was associate with mitochondrial cardiomyopathy, has variable onset (including intrauterine or neonatal forms), phenotypes and severity. Marchet et al. (13) reported two unrelated adult patients from consanguineous families with homozygous mutations in C1QBP were reported. They presenting with progressive external ophthalmoplegia (PEO), mitochondrial myopathy and without any heart involvement.

Herein, we report a homozygous mutation in *C1QBP* caused COXPD 33 in two Chinese brothers. The brothers had an early onset COXPD 33 with clinical manifestations of hypertrophic cardiomyopathy (HCM), exercise intolerance, and documented patterns of increased lactate.

METHODS

Patients and Clinical Investigation

The clinical evaluation was conducted in accordance with the principles of the Declaration of Helsinki. The study was approved by the ethics committee of Xi'an Children's Hospital, the affiliated Children's Hospital of Xi'an Jiaotong University in China. Informed written consent was obtained from all the members of two generations in a Chinese family (**Figure 1**). In the cases of children with ages $<$ 16 years, written informed consent was obtained from the parents. All evaluations included the medical history, family history, physical and laboratory examinations, 12-lead electrocardiographs (ECGs), ultrasonic cardiograms (UCGs), and magnetic resonance imaging (MRI) data.

According to the latest guidelines (14), the clinical diagnosis of HCM was established when the maximum left ventricular wall thickness (MLVWT) $\geq$ 15 mm in adults. For children with ages $<$ 18 years, the diagnosis of HCM required a wall thickness $\geq$ 2 standard deviations (SD) above the predicted mean (Z-score $\geq$ 2) for age, gender, and body size.

Genetic Analysis

Genomic DNA was extracted from 3 mL of whole blood with a blood genomic DNA Mini Kit (CW2087M, CWBIO, Beijing, China). Whole exome were captured (SureSelect Human All Exon V6, Agilent, USA) and sequenced on Illumina Nova Seq sequencing platform (Nova seq 6,000, Illumina, USA). Alignment and variant calling were performed with an information technology, platform-specific pipeline software (Torrent Suite, version 4.2) with the plug-in "variant caller" program (Life Technologies). Variants with (a) a minor allele frequency $<$ 0.05 in population databases, such as 1,000 genome, ExAC, ESP, and GnomAD databases, or (b) those which were present in the Human Gene Mutation Database were included in the analysis. The identified mutation was verified among the remaining family members by Sanger sequencing. The copy number variation analysis was predicted based on whole exon next-generation sequencing data. Mitochondrial DNA was extracted from 3 mL of whole blood with a mitochondrial DNA isolation kit (K280-50, Biovision, America). The experiment of long-PCR amplification of mitochondrial DNA was performed in Fulgent Gene Biotechnology Co., Ltd. The American College of Medical Genetics and Genomics standards and guidelines were followed in this study for the interpretation of sequence variants (15).

Structure Prediction of the Protein Encoded by the C1QBP- P.Leu275Phe Mutation

The three-dimensional (3D) structure of the protein encoded by C1QBP- P.Leu275Phe was analyzed using the wild type of C1QBP (PDB accession codes 1P32, https://www.rcsb.org/structure/1P32) and the SWISS-MODEL (http://swissmodel.expasy.org/). Images were acquired with the PyMOL molecular graphics system (PyMOL, https://pymol.org/2/).

RESULT

Clinical Characteristics

The proband was a 14-year old boy. Birth weight, length, and head circumference were within normal limits. His early milestone acquisitions were appropriate for his age. He presented with systemic edema and admitted to the local hospital when he was one and a half years. The UCG examination revealed left ventricular hypertrophy and ventricular endocardium (END) thickened with endocardial fibroelastosis (EFE) was diagnosed. After symptomatic treatment, the edema was reduced. He was then discharged with continuous oral administration of captopril 6.25 mg/24 h, digoxin 0.0625 mg/48 h, and prednisone 5 mg/48 h. Approximately 7 years later, he stopped captopril. Meanwhile, the dosage of digoxin was weaned from 0.0625 to 0.0417 mg/48 h and the dosage of prednisone was increased from 5 to 10 mg/48 h. The regular UCG examination showed that the thickness of left ventricular posterior wall (LVPW) and interventricular septum (IVS) were increased from 9 to 14 mm and 8 to 9 mm, respectively. Thickness of END was about 2.1–3.5 mm, without significant thicken compared with that of onset time. Furthermore, exercise intolerance with fatigue developed

gradually. The patient was referred to the pediatric cardiology department of Xi'an Children's hospital at the age of 14. Physical examination revealed the boy is 132.5 cm tall and weighs 24.5 kg, slack skins, upturned nose, and ptosis (with an ∼50% coverage of the cornea). He had a blood pressure of 100/58 mmHg with a regular pulse rate at 86 beats per min (bmp). He was afebrile at

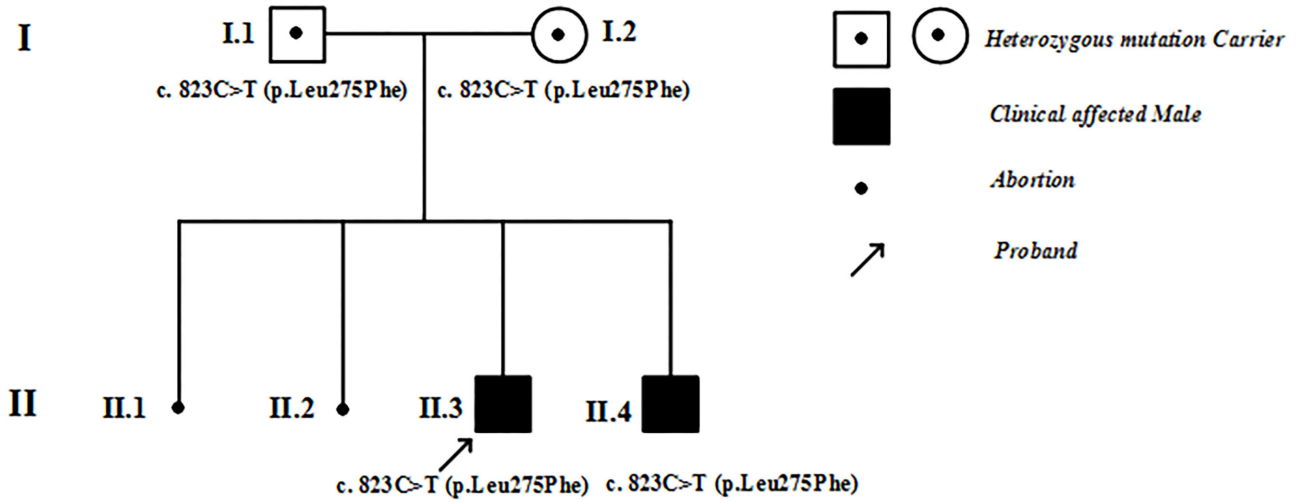

FIGURE 1 | Family pedigree. I, II refer to the first and second generations in this family. Square symbols represent males, circles denote females. Filled black symbols represent patients with hypertrophic cardiomyopathy, and symbols with dots represent mutation carriers without clinical manifestations. The black spots denote abortions. The arrows indicate the proband of this family.

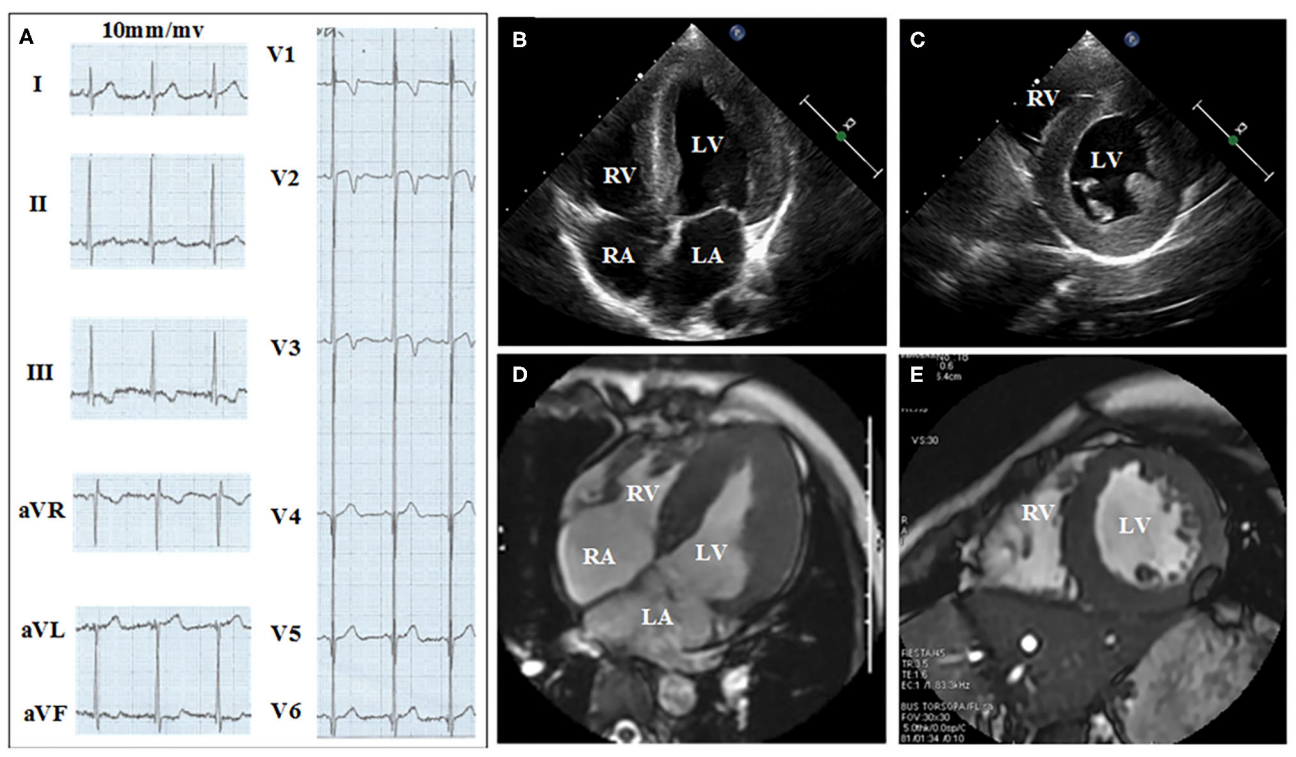

FIGURE 2 | Electrocardiograph (ECG), ultrasonic cardiogram (UCG), and Magnetic Resonance Imaging (MRI) results acquired from the proband show the thickness of the septum and the left ventricular. **(A)** Twelve-lead ECG strips; UCG images of proband in **(B)** four-chamber and **(C)** short-axis views; MRI of proband in **(D)** four-chamber and **(E)** short axis views.

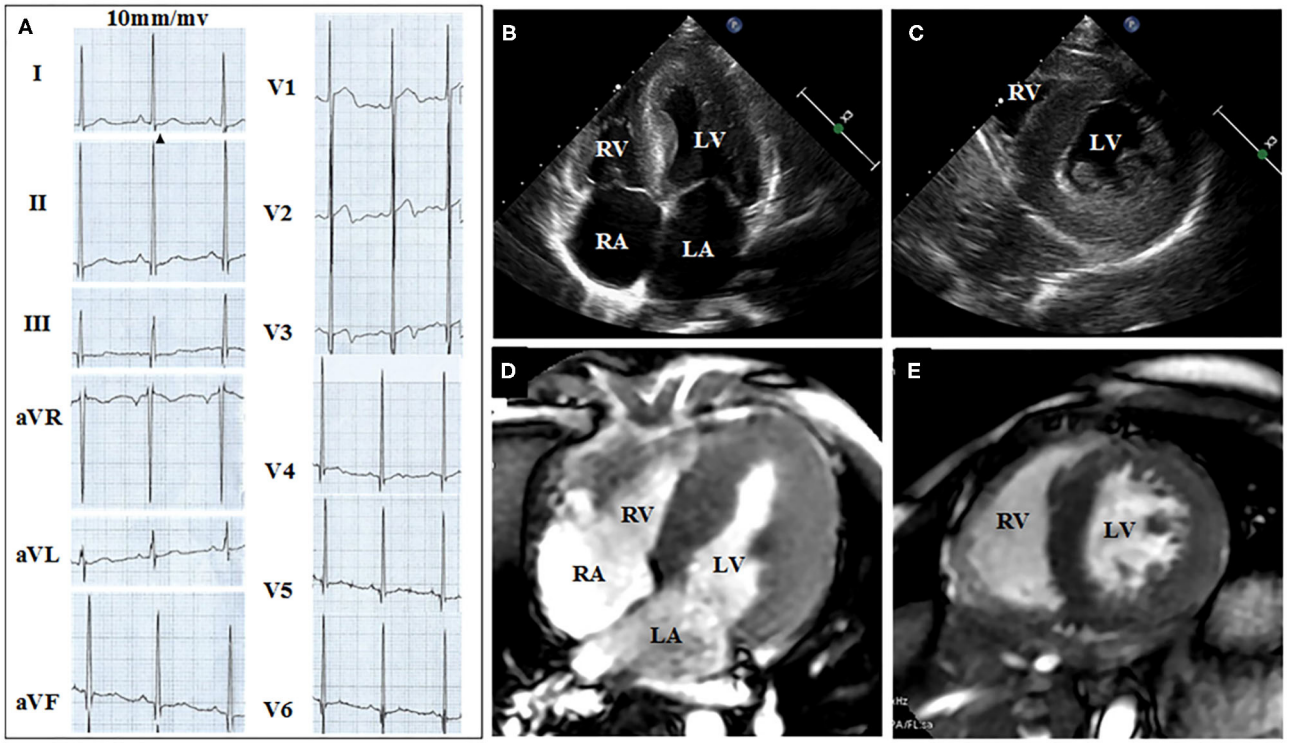

FIGURE 3 | ECG, UCG, and MRI of the younger brother show the thickness of septal and the left ventricular wall. **(A)** Twelve-lead ECG strips; UCG images of brother in **(B)** four-chamber and **(C)** short-axis views; MRI of brother in **(D)** four-chamber and **(E)** short-axis views.

36.7°C, and had a respiratory rate of 24 times/min. And breath sound was clear without wet and dry rales in both lungs. There was no bulge or tremor in the precordial region, and percussion heart boundary enlarged to the left. A regular heartbeat at 86 bpm, and heart sound was strong. No obvious murmur was detected. The abdomen was flat and soft, and there were no palpable enlargements of the liver, spleen, and there were no abnormalities of the nervous system.

The proband's brother, who is a 9-year-old boy. Birth weight, length, and head circumference were also within normal limits. He was admitted to a local hospital with pneumonia when he was 2 years old. The UCG examination showed that the thickness of END significantly increased to ∼5.1 mm. He was also diagnosed with EFE. Diuretics and myocardial nutrients were administered. He also received orally captopril, digoxin, and prednisone at the same dosages as those administered to his brother. The UCG results showed that the thickness of the IVS and the LVPW endocardium gradually thickened from 7 to 9.7 mm, and from 9 to 13.7 mm, respectively. The thickness of the END appeared to recover from 5.1 to 3.9 mm compared with the initial recording. Gradually, he also developed exercise intolerance. He was referred to our hospital together with his brother. Physical examination showed that he had a blood pressure of 100/60 mmHg with a regular pulse rate at 90 bpm. He was afebrile at 36.6°C, and had a respiratory rate of 24 times/min. His lung breath sounds were clear, no bulge or tremor in the precordial region, and the lower left side of the heart is enlarged. A regular heartbeat at 90 beats per min, and heart sound was strong. No murmur was detected in each auscultatory valve areas. The abdomen was flat and soft. Additionally, there were no palpable enlargements of the liver, spleen, and there were no abnormalities found in the nervous system.

Figure 1 shows the pedigree of the family. The proband (II-3) and his brother (II-4) were born after uneventful pregnancies to Chinese parents. Their mother had two spontaneous abortions at 8 weeks of pregnancy before she gave birth to the proband.

Figures 2, **3** show the results of ECG, UCG, and MRI. The ECG of the proband (**Figure 2A**) and his younger brother (**Figure 3A**) showed the voltages of QRS wave were increased significantly (leads V1–V6) that were indicative of cardiac hypertrophy. The UCG results of the proband (**Figures 2B,C**) showed that the thicknesses of LVPW, IVS, and END, were ∼14, 9, and 2.1–3.5 mm, respectively. The UCG results of the younger brother (**Figures 3B,C**) show that the thicknesses of LVPW, IVS, and END, were 13.7, 9.7, and 1.9–3.9 mm, respectively. These findings were consistent with the MRI scan (**Figures 2D,E** for the proband, **Figures 3D,E** for the younger brother). Thus, the two brothers were diagnosed with left ventricle hypertrophy. And their parents were asymptomatic without reported syncope or cardiac arrest.

Table 1 summarizes the clinical characteristics and the laboratory test results of the two brothers. The blood lactate and ammonia in two brothers were higher than normal limits (**Table 1**). The concentrations of the N-terminal probrain

natriuretic peptide (NT-proBNP) and high-sensitive cardiac troponin (hs-TnT) were increased in the two brothers, which suggesting a cardiac functional damage. The GC-MS analyses of organic acids in the urine showed that the values for lactic-2, 2-OH-isovaleric-2, 4-OH-phenyllactic (PHPLA)-3, 2-Keto-isovaleric-OX-2, 2-K o-isocaproic-OX-2, and 2-Keto-3-Methylvaleric-OX-2 were increased (**Table 1**). The remaining organic acids in the urine were normal (**Supplementary Figure 1**). The serum free fatty acid acyl-carnitine analysis using HPLC-QQQ-MS were normal (**Supplementary Table 1**). There were no significant abnormalities in liver function, blood glucose, and myocardial enzymes—including aspartate aminotransferase (AST), creatine kinase (CK), creatine kinase-MB (CK-MB), α-hydroxybutyrate dehydrogenase (α-HBDH), lactate dehydrogenase and its isoenzyme (LDH and LDH-1)—in these two brothers.

Genetic and Bioinformatics Analysis

Figure 4 showed electrophoresis results of amplified mitochondrial DNA by long-PCR, and none large-scales deletion of mitochondrial DNA was detected in both patients and their parents.

The proband's genetic testing identified a homozygous mutation c.823C>T (**Figure 5A**,a) in exon 6 of the C1QBP gene (NM_001212.3) inherited from the mother (**Figure 5A**,c) and father (**Figure 5A**,d) in an autosomal recessive pattern. The parents were asymptomatic heterozygous mutation carriers. And the brother' mutation type (**Figure 5A**,b) was the same as proband. This mutation resulted in the substitution of leucine with phenylalanine at codon 275 (p. Leu275Phe) of the C1QBP protein (**Figure 5B**), denoted as C1QBP- p.Leu275Phe.

C1QBP- p.Leu275Phe has not been presented previously in 1,000 genome, ExAC, ESP and GnomAD databases. The pathogenicity of C1QBP- p.Leu275Phe was predicted to be damaging using multiple software programmes. Therefore, the variant of C1QBP- P.Leu275Phe are defined as likely pathogenic variants according to the ACMG guidelines. No abnormal copy number variations were found based on copy number variation analysis of whole exon next-generation sequencing data. And a variant list contains the genes that contained likely benign mutations and variants of unknown significance were showed in **Supplementary Table 2**.

Human C1QBP forms a homotrimer arranged in a doughnut-shaped structure with an unusually asymmetric charge distribution on the surface (8). There are three C1QBP molecules in an asymmetric unit that form a tightly bound trimer (**Figure 5C**,a). The visualization of the overall architecture shows that the β-sheets form a hyperboloid-shaped spool with the α-helices wrapped around it. Structural analyses of 3D model show that p. leu275 was localized in the αC helix of the protein (**Figure 5C**,b). The p. Phe275 of the mutation type is shown in **Figure 5C**,c.

DISCUSSION

In this study, we reported an early onset of COXPD 33 in two Chinese brothers with HCM, exercise intolerance,

TABLE 1 | Genetic and clinical findings in individuals with C1QBP mutation.

	Proband (II-3)	Brother (II-4)
Basic information		
Gender	Male	Male
Age of onset	1 and a half years old	2 years old
Current age	14 years old	9 years old
Site of mutation		
C1QBP variant (GenBank: NM_001212.3)	c. 823C>T (p. Leu275Phe)	c. 823C>T (p. Leu275Phe)
Physical examination		
Height	132.5 cm	124.5 cm
Weight	24.5 kg	23 kg
Exercise tolerance	Decreased	Decreased
Ptosis	Noticeable	Unapparent
Lab examinations		
Blood lactate	2.58 mmol/L (0.7–2.1)	3.29 mmol/L (0.7–2.1)
Blood ammonia	42.34 μmol/L (9–30)	20.92 μmol/L (9–30)
Blood glucose	4.36 mmol/L (3.5–5.6)	4.72 mmol/L (3.5–5.6)
NT-proBNP	871.30 pg/ml (<300)	428.80 pg/ml (<300)
hs-TnT	14.97 pg/ml (0–14)	24.07 pg/ml (0–14)
Urine organic acid analysis		
Lactic-2	66.80 (0.00–6.70)	452.48 (0.00–6.70)
2-OH-isovaleric-2	6.62 (0.00–0.50)	19.18 (0.00–0.50)
4-OH-phenyllactic (PHPLA)-3	114.15 (0.00–12.51)	313.75 (0.00–12.51)
2-Keto-isovaleric-OX-2	1.16 (0.00–0.50)	3.44 (0.00–0.50)
2-Keto-isocaproic-OX-2	2.90 (0.00–0.73)	8.94 (0.00–0.73)
2-Keto-3-methylvaleric-OX-2	1.61 (0.00–0.50)	4.10 (0.00–0.50)
UCG		
LVIDd	3.8 cm	3.5 cm
LVIDs	2.6 cm	2.1 cm
LA	22 mm	21 mm
LA (Length/width)	27/18 mm	32/27 mm
RA (Length/width)	30/29 mm	29/28 mm
LV (Length/width)	57/31 mm	59/36 mm
RV (Length/width)	49/30 mm	48/25 mm
Thickness of END	2.1–3.5 mm	1.9–3.9 mm
Thickness of IVS	9 mm	9.7 mm
Thickness of LVPW	14 mm	13.7 mm
EF	60%	68%
MRI		
Short-Axis view	LVTDd	38.0 mm	31.2 mm
	LVTDs	15.4 mm	11.5 mm
	Thickness of LVWd	7.38 mm	7.2 mm
	Thickness of LVPWs	19.5 mm	20.9 mm
Four-Chamber view	LVLDd	67.8 mm	66.3 mm
	LVLDs	52.4 mm	50.4 mm
	Thickness of IVSd	14.1 mm	15.1 mm
	Thickness of IVSs	16.5 mm	21.1 mm
LVOT diameter	15.5 mm	13.0 mm	

(s, end-systolic; d, end diastolic) LVID, left ventricular internal dimension; LAID, left atrium internal dimension; LA, left atrium; RA, right atrium; LV, left ventricular; RV, right ventricular; END: endocardium; IVS, interventricular septum; LVPW, left ventricular posterior wall; EF, ejection fraction; LVTD, left ventricular internal trans dimension; LVWd, Thickness of left ventricular wall; LVLW, Thickness of left ventricular lateral wall; LVLD, left ventricular long dimension; LVOT, left ventricular out flow tract.

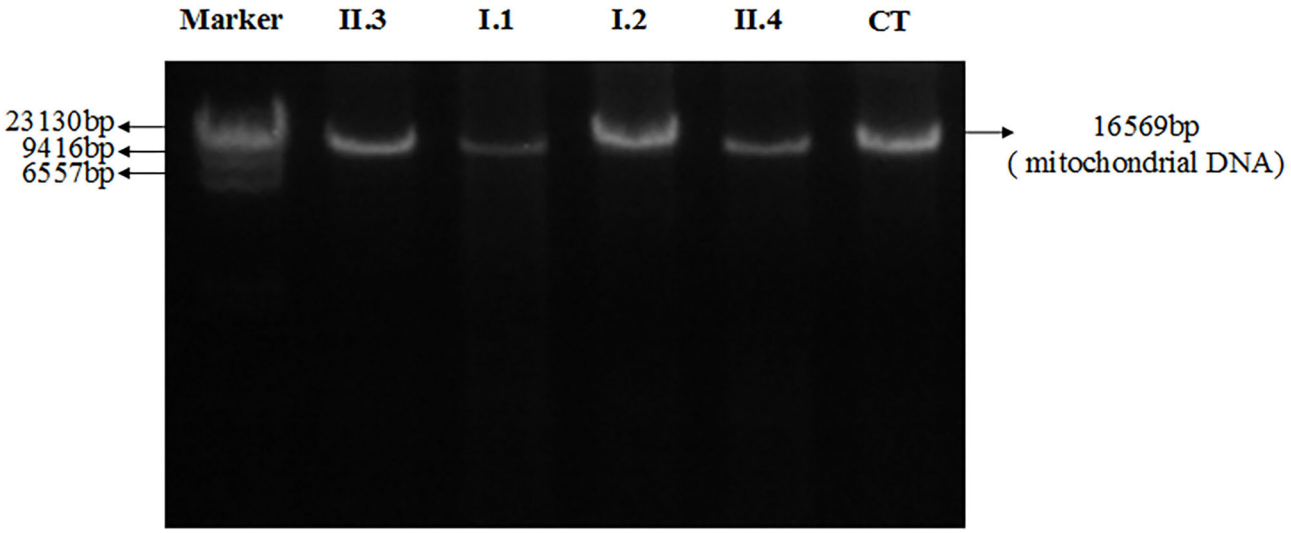

FIGURE 4 | Electrophoresis results of amplified mitochondrial DNA by long-PCR of all patients and their parents. II.3: Proband; I.1: Father; I.2: Mother; II.4: Brother; CT: Control.

and increased lactate caused by the homozygous C1QBP-P.Leu275Phe mutation for the first time. The homozygous C1QBP- P.Leu275Phe mutation resulted in a defect in mitochondrial energy metabolism. HCM is one of the most common and important of cardiac phenotype associated with mitochondrial respiratory disorders (16, 17). Furthermore, the mitochondrial respiratory disorders is involves the development of heart failure (18, 19).

Prior research has indicated that a p32 mutation was the suspected cause of mitochondrial respiratory chain disorders (20). Four individuals from unrelated families with biallelic mutations in *C1QBP* had COXPD 33 (12). They were presented with exercise intolerance, progressive external ophthalmoplegia (PEO), and cardiomyopathy.

The onset age of the disease was variable. C1QBP-p.Gly247Trp and the p.Leu275Phe compound mutation caused intra-uterine neonatal cardiomyopathy. While the combinations of C1QBP-p.Cys186Ser and p.Pro204Leu mutation led to early death. The symptom onset of the homozygous mutation of C1QBP- p.Tyr188del was in adulthood. The only alive patient carried homozygous mutation of C1QBP- p.Leu275Phe. The diseases onset of this patient was in 5 years old with the increase of serum lactic acid, creatine kinase, transaminase, methionine and tyrosine. The clinical symptoms include left ventricular hypertrophy, fatiguability and ptosis. The primary clinical sign and symptoms were in line with our report. While the disease onset age in our study started from infancy which was earlier than that in the reported literature.

Feichtinger et al. (12) have also presented the functional studies muscular enzymology research. The functional studies of C1QBP- p.Leu275Phe mutation indicated that C1QBP protein could not be detected in the biopsy muscle of the patient, and C1QBP protein was significantly reduced in fibroblasts (primary fibroblast culture of the patient). Muscular enzymology studies showed a corresponding decrease in complex I and complex IV subunits, leading to mitochondrial respiratory chain defects.

Furthermore, two unrelated adult patients with homozygous mutations in C1QBP were reported (13). They presenting with progressive external ophthalmoplegia (PEO), mitochondrial myopathy and without any heart involvement. Muscle biopsies from both patients showed typical mitochondrial alterations and the presence of multiple mitochondrial DNA deletions. While in our study, the patients only have ptosis without any other eye problems. And none large-scales deletion of mitochondrial DNA was detected in both patients and their parents.

In addition, the mother of the proband in our study had two spontaneous abortions at 8 weeks of pregnancy before she gave birth to the proband. According to a prior report [11], C1QBP-deficient mice exhibited embryonic lethality. We suspect that the reason for the abortion was related to a C1QBP-deficiency. However, the exact cause of the spontaneous abortion could not be confirmed because autopsies were not performed in the cases of these two aborted fetuses.

The wild type (p. Leu275) and mutation type (p. Phe275) are localized in the αC helix of the C1QBP protein. The αC helix is an important structural domain of the protein. The N-terminal portion of the helix αC and Helix αB make extensive hydrophobic contacts with the β-sheet that are essential for the stability of the structure. Furthermore, the C-terminal portion of αC forms an antiparallel coiled-coil with the N-terminal helix αA. This coiled-coil region is important for protein–protein interactions and is responsible for homo-oligomerization (8). The amino acid residues of L and F are all nonpolar amino acids and have similar isoelectric points. This indicates that the properties of the amino acids do not influence the structure and function of C1QBP. The mutation C1QBP- P.Leu275Phe may

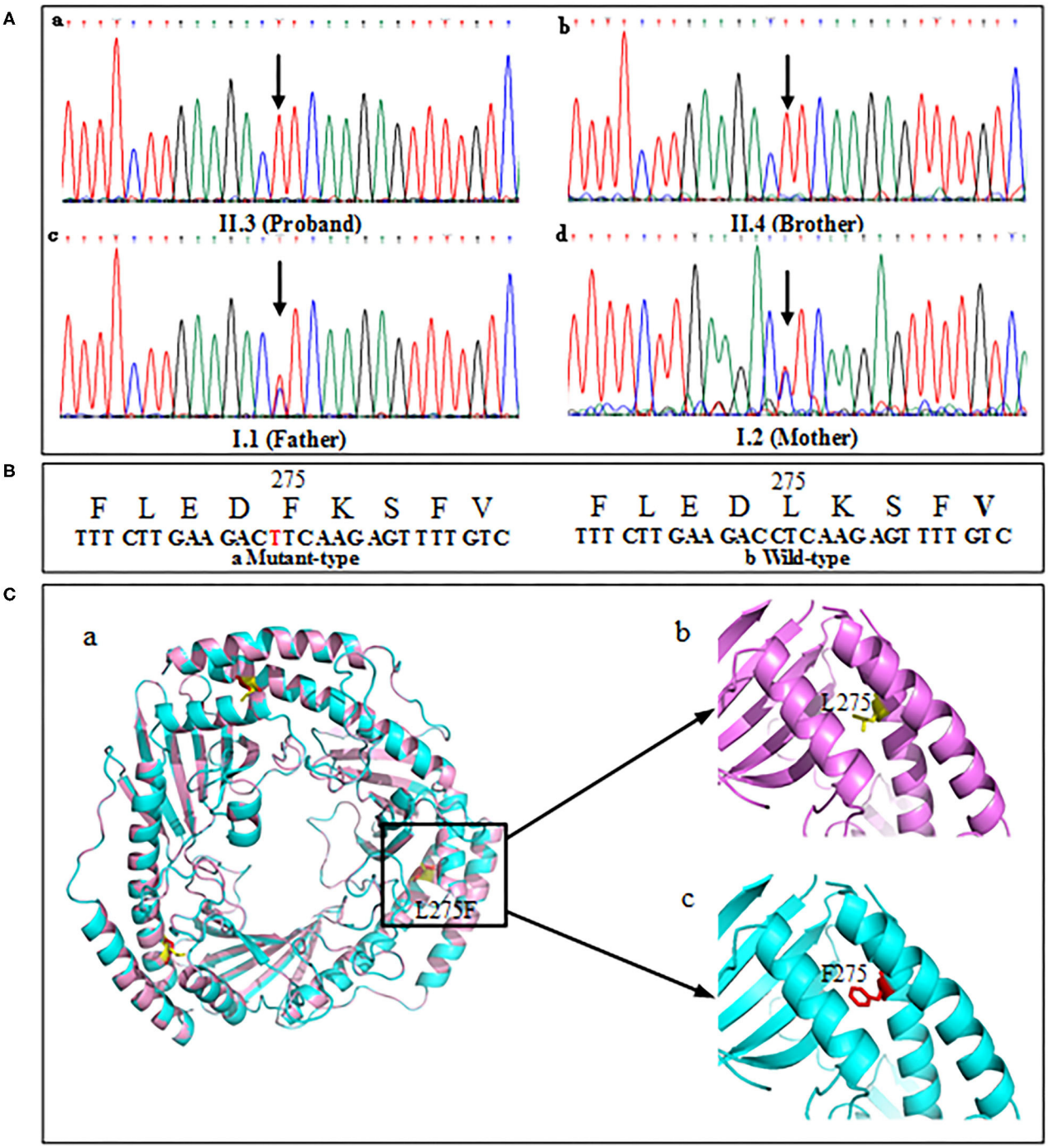

FIGURE 5 | Mutation screening of tested family. **(A)** DNA changes identified based on sequencing. **(B)** Partial amino-acid sequence of wild-type (left) and mutant (right) were deduced. **(C,a)** Predicted three-dimensional structure of the C1QBP-L275F protein (PDB accession codes 1P32, https://www.rcsb.org/structure/1P32). **(C,b)** Wild type, L275 is shown in yellow color. **(C,c)** Mutation type, F275 is shown in red color.

affect the localization of the αC helix domain, and may thus increase the exposure of the hydrophobic surface. Furthermore, the coiled-coil regions of αA and αC were reported to form extensive intermolecular contacts (8). The mutation of the 275 amino acid residue in the αC helix domain may also influence the assembly of the C1QBP subunits. Thus, the preliminary conclusion is that the mutation C1QBP- P.Leu275Phe in the αC helix domain may lead to a faulty function of C1QBP owing to

its effects on the localization of the αC helix domain, increase the exposure of the hydrophobic surface, or influence the assembly of subunits.

LIMITATION

There are a few limitations in this study. First, we did not get the permission from the parents for muscle biopsy. Therefore, the C1QBP- p.Leu275Phe variant functional studies, muscular enzymology and histological/histochemical experiments were not performed. Secondly, the deep intronic mutations as well as complex indels deep intron variant could not been exclude using Next-generation sequencing.

CONCLUSIONS

We demonstrated the clinical consequences of COXPD 33 caused by homozygous C1QBP- P.Leu275Phe mutations in autosomal recessive inherited mode in two Chinese brothers with early onset since infancy. The phenotypes were characterized by HCM, exercise intolerance, and increased lactate levels.

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

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

INFORMED CONSENT

Informed written consent was obtained from all the members of two generations in a Chinese family. In the cases of children with ages < 16 years, written informed consent was obtained from the parents.

AUTHOR CONTRIBUTIONS

YZ: conceptualization. YZ, JW, HL, and MS: methodology. JW and QY: software. YY, BL, FL, and WH: validation. JW and YZ: formal analysis. JW, YZ, MS, and HL: writing original draft preparation. YZ, JW, HL, and MS: writing review and editing. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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ORIGINAL RESEARCH
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Differential Expression Profiles and Functional Analysis of Long Non-coding RNAs in Children With Dilated Cardiomyopathy

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Aim: To evaluate the expression profile of long non-coding RNAs (lncRNAs) in different left ventricular function of dilated cardiomyopathy (DCM) in children and explore their possible functions.

Methods: The lncRNA microarray experiment was used to determine the differential expression profile of lncRNA in three children with DCM and three healthy volunteers. The functional analysis and the construction of the lncRNA-mRNA interaction network were carried out to study the biological functions. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was used to verify the microarray data.

Results: There were 369 up-regulated lncRNAs identified in the DCM patients (fold change >2, $P < 0.05$), and 505 down-regulated lncRNAs. Based on target gene prediction and co-expression network construction, 9 differentially expressed lncRNAs were selected for the PCR to verify the accuracy of the microarray data, of which 5 were up-regulated and 4 were down-regulated, and finally proved that 7 of them were consistent with the trend of microarray data results. Four of these lncRNAs had significant differences between the patients with poor cardiac function and patients with improved left ventricle function.

Conclusion: LncRNAs may play an important role in pediatric DCM and may provide a new perspective for the pathogenesis, diagnosis, and treatment of this disease.

Keywords: pediatric dilated cardiomyopathy, long non-coding RNAs, Microarray, gene expression profile, functional analysis, biomarkers

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INTRODUCTION

Dilated cardiomyopathy (DCM) is the main cause of progressive refractory heart failure which necessitates heart failure and can lead to death (1). DCM is defined by the presence of a dilated left ventricle with systolic dysfunction in the absence of a hemodynamic cause which can lead to dilation and dysfunction (2). The annual incidence of DCM in children accounts for approximately 50% of pediatric cardiomyopathies (0.57/100,000) and is the most common type of pediatric cardiomyopathy (3, 4). The mortality rate of children with DCM is high, and most children with this condition die from heart failure (5). High-risk factors of children with DCM include decreased left ventricular function, left ventricular dilation, and left ventricular posterior wallthinning (6).

TABLE 1 | Genes associated with familial dilated cardiomyopathy.

Gene	Protein	Mutation Frequency (%)
TTN	Titin	20–25
LMNA	Lamin-A/C	5
MYH7	Myosin-7 (β-myosin heavy chain)	4
MYH6	Myosin-6 (α-myosin heavy chain)	4
TNNT2	Cardiac muscle troponin T	2
MYBPC3	Cardiac-type myosin-binding protein C	2
MYPN	Myopalladin	2
SCN5A	Sodium channel protein type 5 subunit α	2
RBM20	RNA-binding protein 20	2
PLN	Phospholamban	1

However, the pathogenesis of DCM remains unclear yet. The existence of family aggregation in DCM suggests that genetics play a certain role in its pathogenesis. In recent years, literature has confirmed that familial DCM accounts for 20–50% of the total number of DCM cases (7). Further, an increasing amount of gene mutations have been found to be highly related to DCM, of which autosomal dominant inheritance accounts for 90% of all gene mutations including 16 genes, the 10 genes with the highest mutation frequency are listed in **Table 1** (8, 9). But in general this may only account for a small part of the genetic cause. Another hypothesis supported by previous studies suggests that failure to eliminate pathogens or activation of chronic autoimmune mechanisms against cardiac antibodies after subacute and chronic viral myocarditis may finally foster the conversion to DCM (10). Therefore, further clarification of its etiology and pathogenesis is very important for clinical and scientific research.

Long non-coding RNA (lncRNA) are usually defined as transcripts greater than 200 nucleotides in length of non-coding RNA (ncRNA). This ncRNA is a type of RNA that can be transcribed from the genome, but not translated into protein (11). LncRNA is comprised of tens of thousands or even hundreds of thousands of nucleotides, so it has an extremely complex secondary structure, and it can perform a variety of molecular functions such as signaling, decoy, guidance, and scaffolding by interaction with DNA, RNA, or proteins (12, 13). They can be located in the nucleus or in the cytoplasm and can be alternatively spliced or polyadenylated (12). It has been reported that lncRNA is indeed regulated in human cardiac diseases. Additionally, there is increasing evidence that lncRNAs play a vital role in vascular biology and cardiovascular diseases, such as myocardial infarction, cardiac hypertrophy and fibrosis, atherosclerosis, angiogenesis, and vascular remodeling (14). These lncRNAs can act as competing endogenous RNAs (ceRNAs) or natural microRNA sponges to bind miRNAs competitively for gene regulation (15) and can regulate co-expressed coding genes to affect gene expression by chromosomal looping (16) or by directly binding chromatin-modifying complexes (17) and transcription factors (18). In addition, the lncRNA Microarray Chip is an effective tool for high-throughput analysis of lncRNA expression and it has been used to study the expression profiles of lncRNA in many kinds of diseases (19). However, data related to DCM in children is sparse, and a few studies have focused on adult DCM and lncRNAs (20, 21).

In order to clarify whether lncRNA in peripheral leukocytes is associated with the occurrence of DCM in children, and if it correlates with progression or improvement of the disease, we used the Microarray Chip to analyze differential expression of lncRNAs and mRNAs in children with DCM and healthy volunteers.

MATERIALS AND METHODS

Patients Selection and Sample Collection

Between March 2019 and December 2019, we selected 25 children with DCM (age 1 month to 17 years) and 25 healthy children at the Department of Pediatric Cardiology, Shandong Provincial Hospital Affiliated to Shandong University. All DCM cases were diagnosed clinically according to the Classification and Diagnosis of Cardiomyopathy in Children as set by the American Heart Association (2). The exclusion criteria were as follows: DCM after chemotherapy; tachycardia induced cardiomyopathy; secondary cardiomyopathy unless secondary to inflammation (alcoholic cardiomyopathy; DCM secondary to the immune system diseases and metabolic or endocrine disease); and DCM combined with other organ system diseases. The control group consisted of healthy children whose age and gender-matched those of DCM children.

We collected peripheral blood (3 mL) from the experimental and control groups, and also collected an additional 20 samples of peripheral blood samples from DCM patients with improved left ventricle function (LVEF $>$ 45%) (22), including samples taken from the same patient during their recovery period. Blood was collected in anticoagulant EDTA tubes and store at 4°C as quickly as possible, generally within 2 h from collection, then white blood cells were separated and frozen into −80°C with TRIzol reagent. The baseline characteristics of the DCM group and the control group, and the clinical characteristics of the DCM group with poor and improved left ventricle function are presented respectively in **Tables 2**, **3**, respectively. Three DCM samples (D1, D2, D3) and three normal samples (C1, C2, C3) were chosen for microarray analysis.

Total RNA Extraction and Purification

Total RNA was isolated using SparkZol Reagent (Sparkjade, Qingdao, China) according to the manufacturer's instructions, and purified by using a RNeasy Mini Kit (Qiagen, GmBH, Germany). Then A NanoDrop ND-2000 spectrophotometer (NanoDrop, DE, USA) was used to check the quantity and purity of RNA. Qualified RNA is used in subsequent experiments such as microarray analysis.

LncRNA Microarray Analysis

RNA samples of DCM and control groups were sent to Shanghai Sinomics Corporation (Shanghai, China) then used to generate biotinylated cRNA targets for the Sino Human ceRNA array V3.0. The slides were then used to hybridize with the biotinylated

TABLE 2 | Detailed information about the DCM patients and controls.

	Median age (y), (Q1, Q3)	Sex		BP (mmHg)		Heart rate (rpm)	Laboratory examination		Echo parameters	
		Male, *n* (%)	Female, *n* (%)	SBP (mmHg)	DBP (mmHg)		Hs-TnT (pg/mL), (Q1, Q3)	BNP (pg/mL), (Q1, Q3)	LVEDD (mm)	LVEF (%)
Healthy children ($n = 25$)	1.75 (0.92, 8.25)	12 (48)	13 (52)	96.08 ± 10.75	64.05 ± 7.16	110.24 ± 17.34	<3	<450	32.19 ± 5.12	64.08 ± 1.52
DCM children ($n = 25$)	1.42 (0.75, 6.75)	10 (40)	15 (60)	92.84 ± 12.1	57.44 ± 11.41	139.8 ± 32.47	42.48 (11.99, 82.36)	9,214 (3213, 16672)	47.7 ± 11.8	30.2 ± 8.6

BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hs-TnT, hypersensitive troponin T (normal range, 3–14 pg/mL); BNP, brain natriuretic peptide (normal range, 0–450 pg/mL); LVEDD, left ventricular end-diastolic dimension, tested by echocardiography; LVEF, left ventricular ejection fraction, tested by echocardiography (normal value >60%).

TABLE 3 | Clinical characteristics about the DCM patients with poor and improved LV function.

	DCM with poor LV function ($n = 25$)	DCM with improved LV function ($n = 20$)
BP (mmHg)		
SBP (mmHg)	92.84 ± 12.1	95.45 ± 9.34
DBP (mmHg)	57.44 ± 11.41	63.1 ± 8.33
Heart rate (rpm)	139.8 ± 32.47	110.8 ± 14.37
NYHA functional class, *n* (%)		
I	–	13 (65)
II	–	5 (25)
III	3 (12)	2 (10)
IV	22 (88)	–
Laboratory examination		
Hs-TnT (pg/mL) (Q1, Q3)	42.48 (11.99, 82.36)	8.45 (4.98, 10.3)
BNP (pg/mL) (Q1, Q3)	9214 (3213, 16672)	121.25 (81.46, 329.75)
Echo parameters		
LVEDD (mm)	47.7 ± 11.8	38.09 ± 13.81
LVEF (%)	30.2 ± 8.6	54.75 ± 7.43

NYHA, New York Heart Association.

TABLE 4 | Primers sequence for quantitative real time-PCR.

Primer name	Primer sequence (5′-3′)	Length (bp)
NONHSAT175499.1	F:TGTGCCTGATATAGTGCTTGGT	93
	R:TCTGTCTTCCTCACTAGCCTGT	
ENST00000560465	F:TGACTGGAAAACCCTCCCCA	128
	R:GCACTCCCACGTCTTATGCTC	
ENST00000596816	F:GCGAAGCTGTCATTAGCAAGG	138
	R:ACACAAACAGCATCCGTCTT	
NONHSAT215378.1	F:CGCAAACAGCAACCATAGCG	106
	R:AGCCTCACTTGGGAAGGAAGT	
NONHSAT252242.1	F:GGCAAGGCATTCTAACCCCAT	197
	R:AGCCCTCCAAGGTGTGTATGA	
NONHSAT137060.2	F:TTGTGAGACACTGGGGAGGT	250
	R:TGTCCTGCCTTTCCACATTCT	
ENST00000457996	F:ACCACGCATCCTGAGACAAAC	213
	R:TAGCCAAACCAACTGCCTCTG	
NONHSAT242978.1	F:CTGCAGCTGTTTCCAAGAGG	71
	R:TGGTAGGAGTCATGGAACCG	
ENST00000637940	F:ACCCCTCTCTCAACTGTCGG	91
	R:TCACTGCCAGAGGTCACCAA	

F, forward; R, reverse.

cRNA targets. After hybridization, slides were scanned on the microarray scanner. Data extraction was performed using feature extraction software 10.7 (Agilent technologies). The Quantile algorithm of the "limma" package in R was used to normalize the raw data. The data analysis was performed in accordance with the protocol specified by Agilent Technologies. A two-fold change cutoff was adopted.

Analysis of RNA Sequencing Data

Genes with at least two-fold change in expression were selected for further analysis. To analyze their function, we predicted the *cis*- and *trans*-target genes of differentially expressed lncRNAs and analog gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichments. The *cis*-target genes were selected based on the genes less than 10 kb away from lncRNA. The *trans*-target genes were predicted by complementary or similar sequences of lncRNA using the National Center for Biotechnology Information basic local alignment search tool (BLAST), the resulting complementary energy between the two sequences was calculated using RNAplex, and the sequences with $e \leq -30$ were selected. The method of GO and KEGG pathway enrichment analyses used Fisher's exact test and the cluster Profiler data package from R were chosen; the selection criterion is the number of genes that fall on fold change ≥ 2, $P < 0.05$, and the term obtained in the drawing is based on the value of the enrichment factor sort in descending order of size. The resulting top 30 pathways were chosen.

In addition, we constructed the lncRNA-mRNA co-expression network using a k-core algorithm to determine which lncRNAs and mRNAs might play a pivotal role in the DCM and the relationship of them. We also constructed lncRNA-miRNA-mRNA network by predicting the miRNA molecules adsorbed by lncRNA.

Quantitative Real Time-PCR

Quantitative real time-PCR (qRT-PCR) was performed by using LightCycler 480 (Roche, Shanghai, China) system to verify the accuracy of the data. The qRT-PCR was conducted according to the instructions of the SYBR® Green Premix Pro Taq

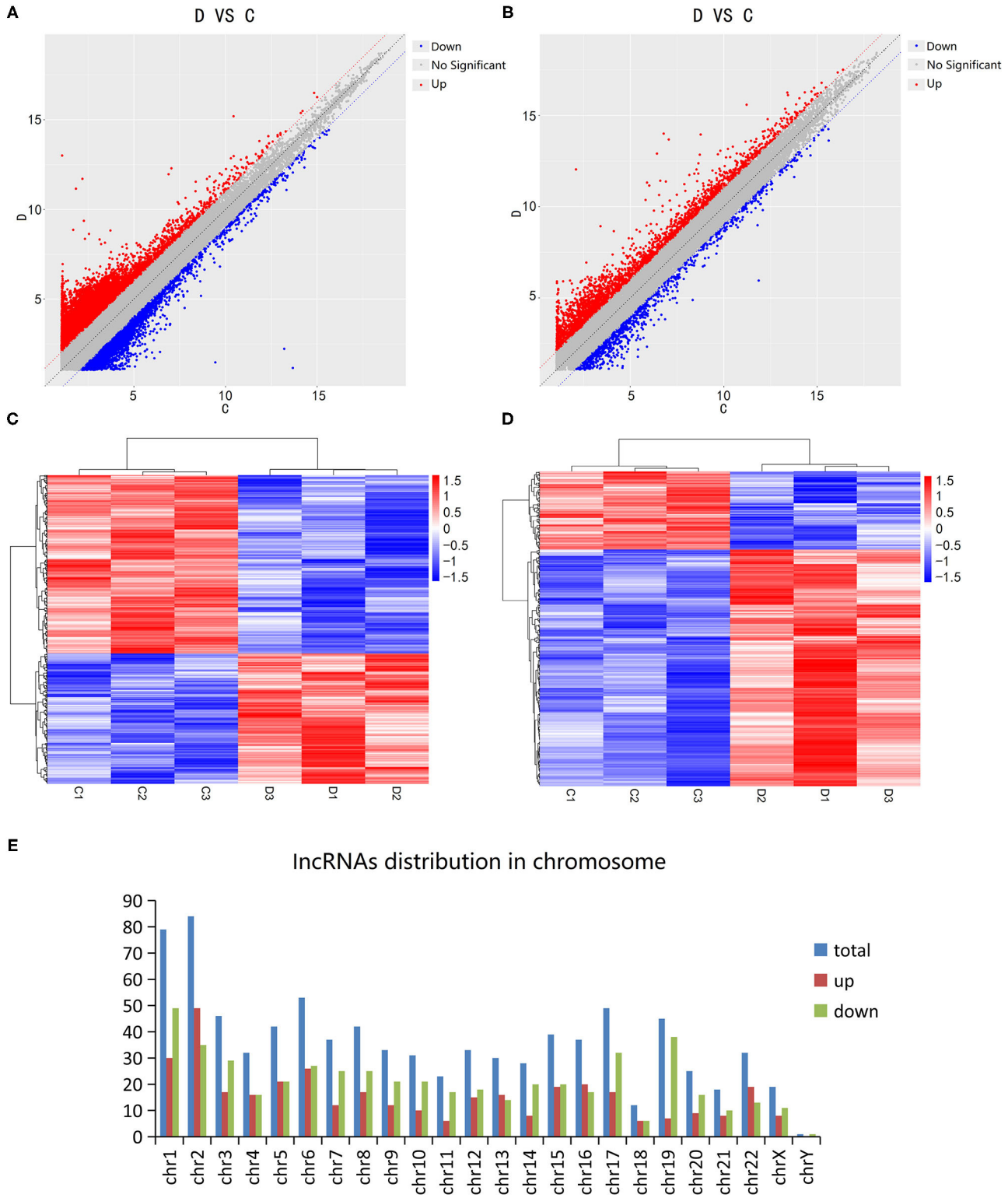

FIGURE 1 | Expression levels of long non-coding RNAs (lncRNA) in dilated cardiomyopathy. Scatter plots were used to distinguish differentially expressed lncRNAs **(A)** and mRNAs **(B)**. Hierarchical cluster analysis of lncRNAs **(C)** and mRNAs **(D)** with altered expression ($P < 0.05$, fold change >2) between the two groups. Distribution of dysregulated lncRNAs in human chromosomes **(E)**. Red and blue represent upregulated and downregulated expression respectively. D indicates the experimental group; C indicates the control group.

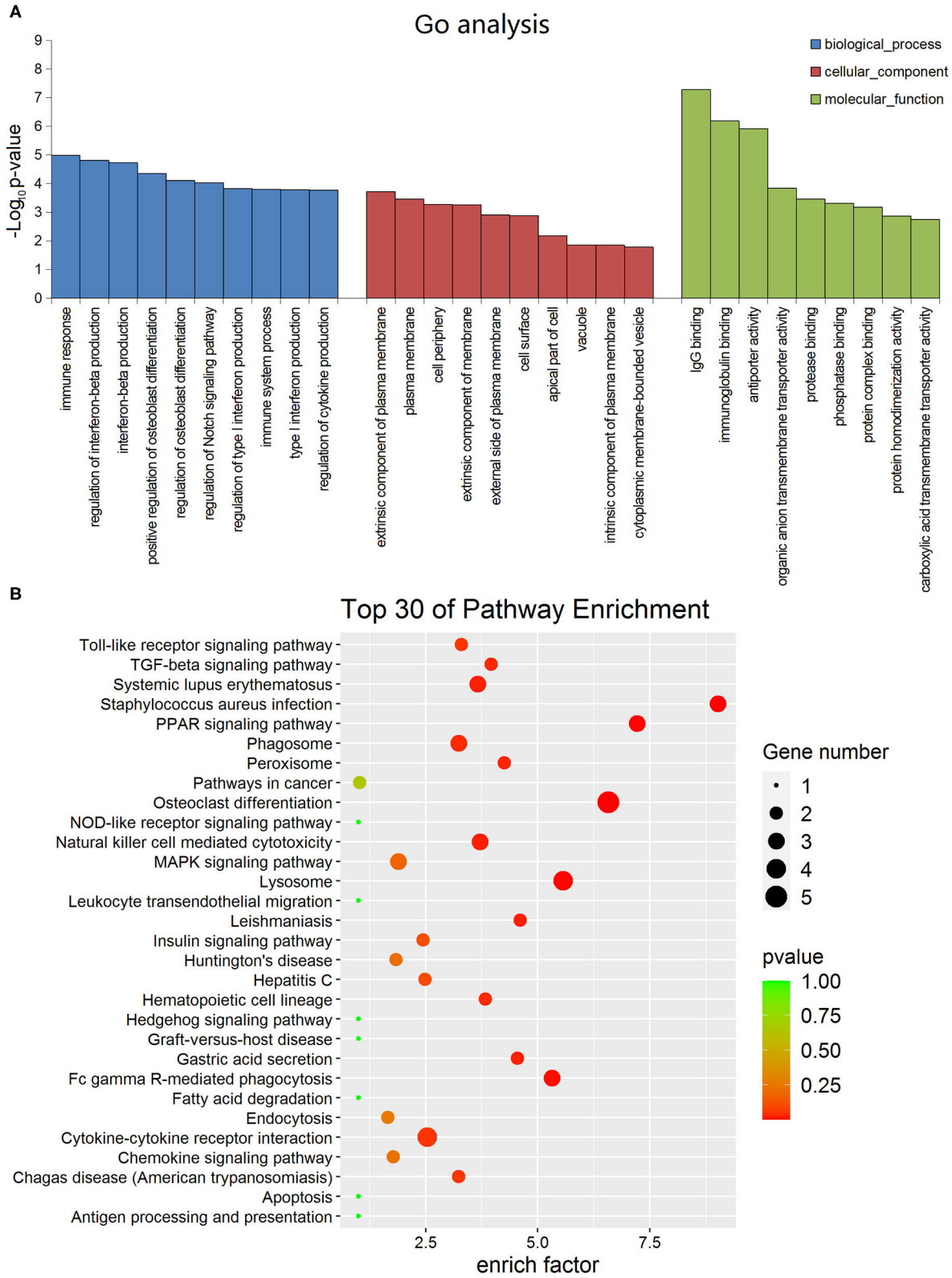

FIGURE 2 | Gene ontology and Kyoto encyclopedia of genes and genomes pathway analyses of dysregulated long non-coding RNA (lncRNA)s in dilated cardiomyopathy. **(A)** The top 10 GO terms of the three different gene functions for lncRNAs. **(B)** The top 30 KEGG pathways for lncRNAs.

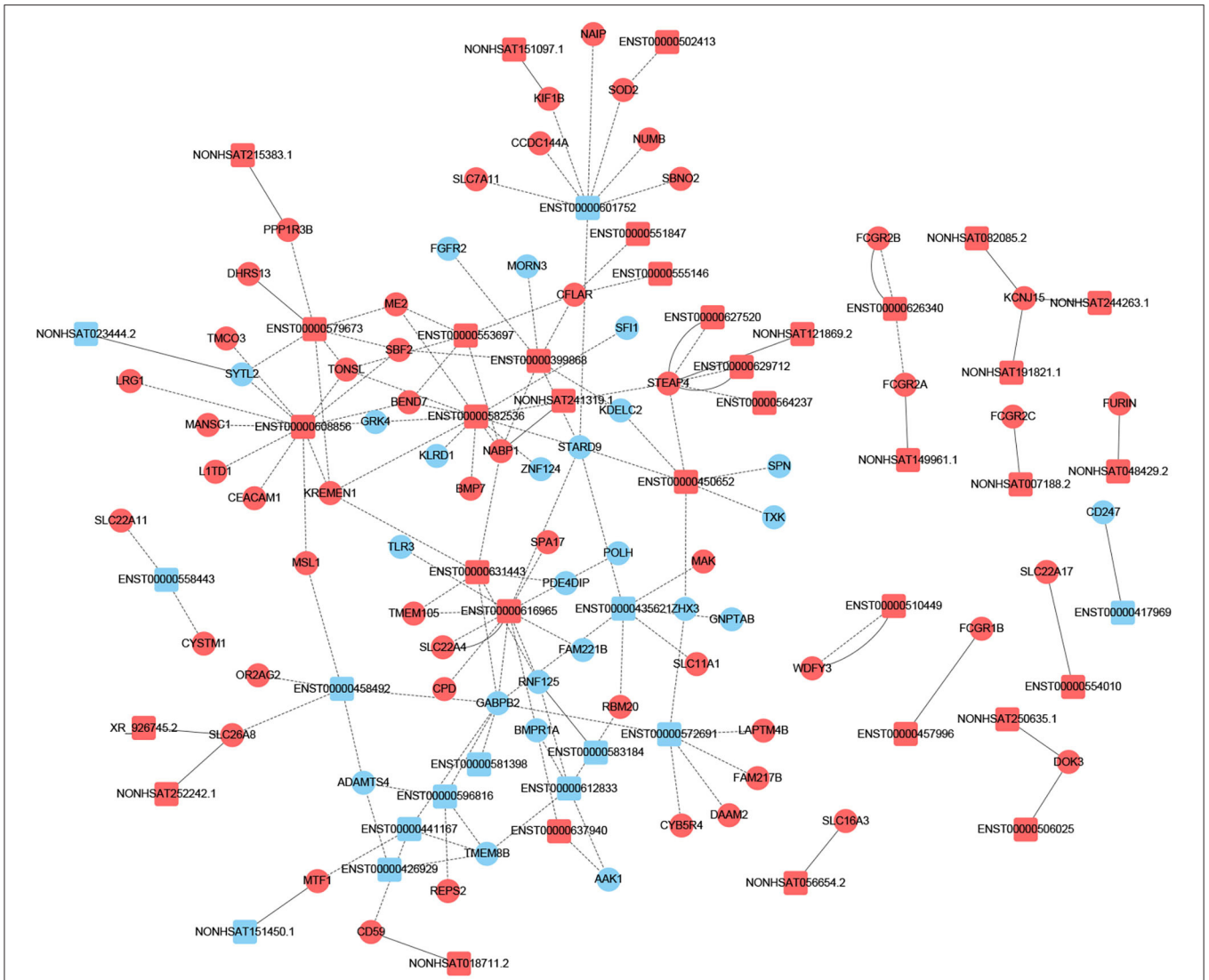

FIGURE 3 | Prediction of *cis*-/*trans*-lncRNA targets in DCM-related pathways. The circles represent mRNAs, and the squares represent lncRNAs. Red means up-regulation, blue means down-regulation. The dotted line indicates a *trans*-lncRNA, and the solid line indicates a *cis*-lncRNA.

HS qPCR Kit (AG11701, ACCURATE BIOTECHNOLOGY, HUNAN) specification. The primer sequences used in the qRT-PCR are illustrated in **Table 4**. The relative expression levels were presented using the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

SPSS25.0 and GraphPad Prism 8.0 were used for data analysis. Continuous data in this article are expressed as mean ± standard deviation (SD), *n* (%) or median, and interquartile range. The difference between each group was compared by Student's *t*-test. $P < 0.05$ was considered as statistically significant.

RESULTS

Expression Profiles of lncRNAs and mRNAs

The Microarray Analysis detected a total of 75,589 lncRNAs in 3 DCM samples and 3 control samples. The differential expression of lncRNA and mRNA in the DCM and control groups are shown as a scatter plot in **Figures 1A,B**. Additionally, the lncRNA and mRNA expression is also shown in a heat map using hierarchical clustering (**Figures 1C,D**). In summary, there are a total of 874 lncRNAs that are differentially expressed in patients with DCM as compared to the control group (fold change >2, $P < 0.05$), of which 369 are up-regulated and 505 are down-regulated. **Figure 1E** shows the distribution of these differentially expressed lncRNAs on human chromosomes. Moreover, there are 641 differentially expressed mRNAs between the DCM group and control group in all, which include 482 up-regulated and 159 down-regulated mRNAs.

GO and KEGG Pathway Analysis

In order to determine the function of these lncRNAs more clearly, we performed GO analysis and KEGG pathway enrichment analysis. The GO enrichment analysis divides the function of genes into three parts: cellular component (CC), molecular

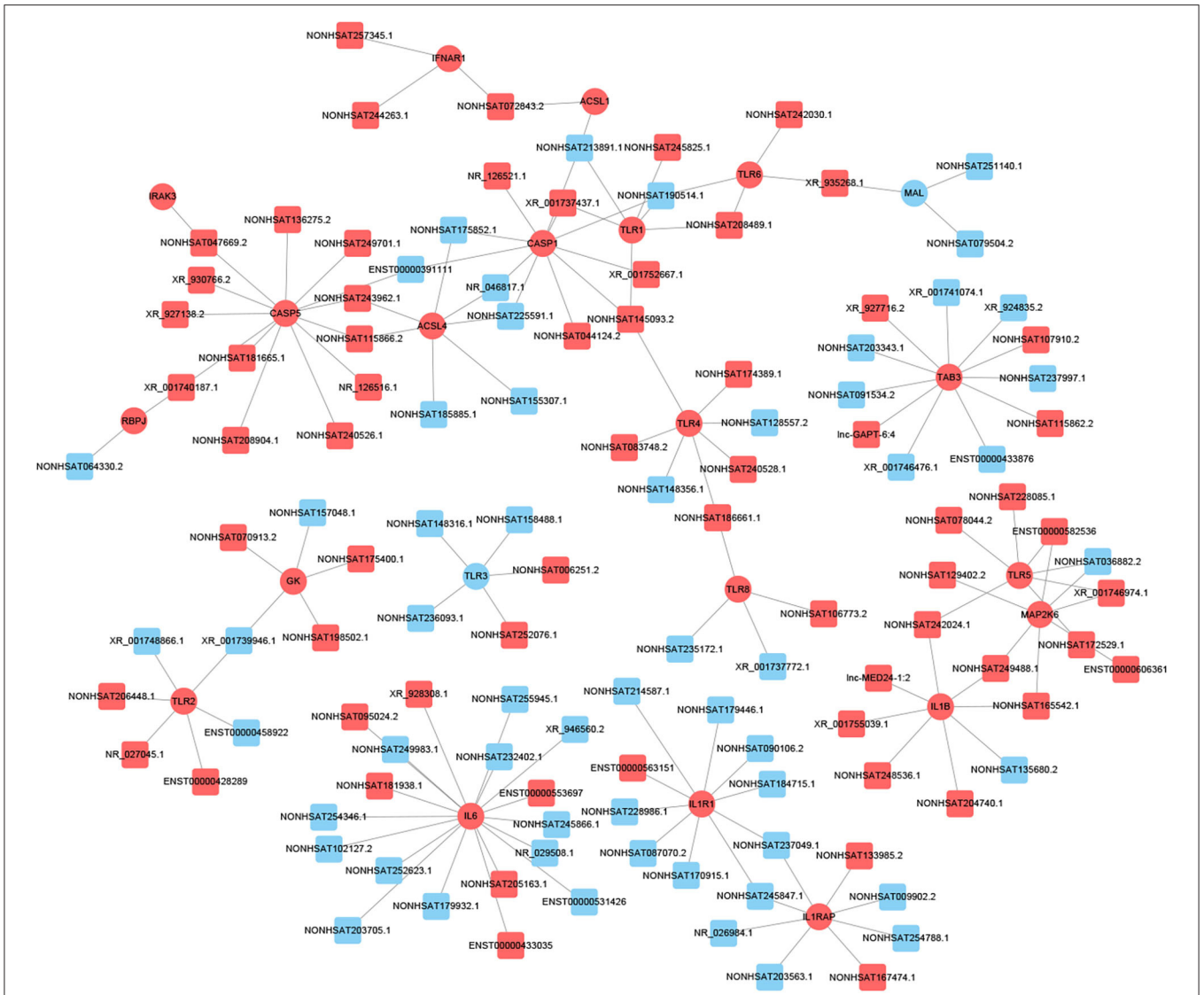

FIGURE 4 | LncRNA-mRNA co-expression network analysis of the DCM-related pathways (Pearson's coefficient > 0.95). The circles represent mRNAs, and the squares represent lncRNAs. Red means up-regulation, blue means down-regulation.

function (MF) and biological process (BP). The top 10 of the three different gene functions of lncRNAs were shown in **Figure 2A**. Immune system and signal transduction are the most noteworthy pathways identified by KEGG classification. We selected the top 30 enriched KEGG pathways in the immune system and signaling pathways as shown in **Figure 2B**. We were more interested in "NF-kappa B signaling pathway," "Toll-like receptor signaling pathway," "PPAR signaling pathway," "Apoptosis," "Notch signaling pathway" and so on as they may be associated with DCM. Then we screened some differentially expressed genes in these pathways for future experiments.

Cis- and *Trans*-lncRNA Target Gene Prediction

To determine if lncRNA could regulate gene expression, we predicted the target genes of lncRNA through different manners of action, including *cis* and *trans*. The principle of *cis*-predicting target genes is that lncRNA regulates the pattern of surrounding mRNA that is relatively close. On the contrary, the basic principle used when predicting *trans*-target genes is that the function of lncRNA has nothing to do with the position of the coding gene, but is related to the protein-coding gene that is co-expressed. We have performed a part of the lncRNA *cis*-/*trans*-target gene prediction in the pathway mentioned above as shown in **Figure 3**. In addition, according to the data, the *cis*-action manner of lncRNA accounts for the majority of the pathway.

Construction of the Interaction Network Between lncRNA and mRNA

Previous studies have shown that lncRNA can act as ceRNAs or natural miRNA sponges to bind miRNAs competitively to reduce the effects of miRNAs on target genes, ultimately

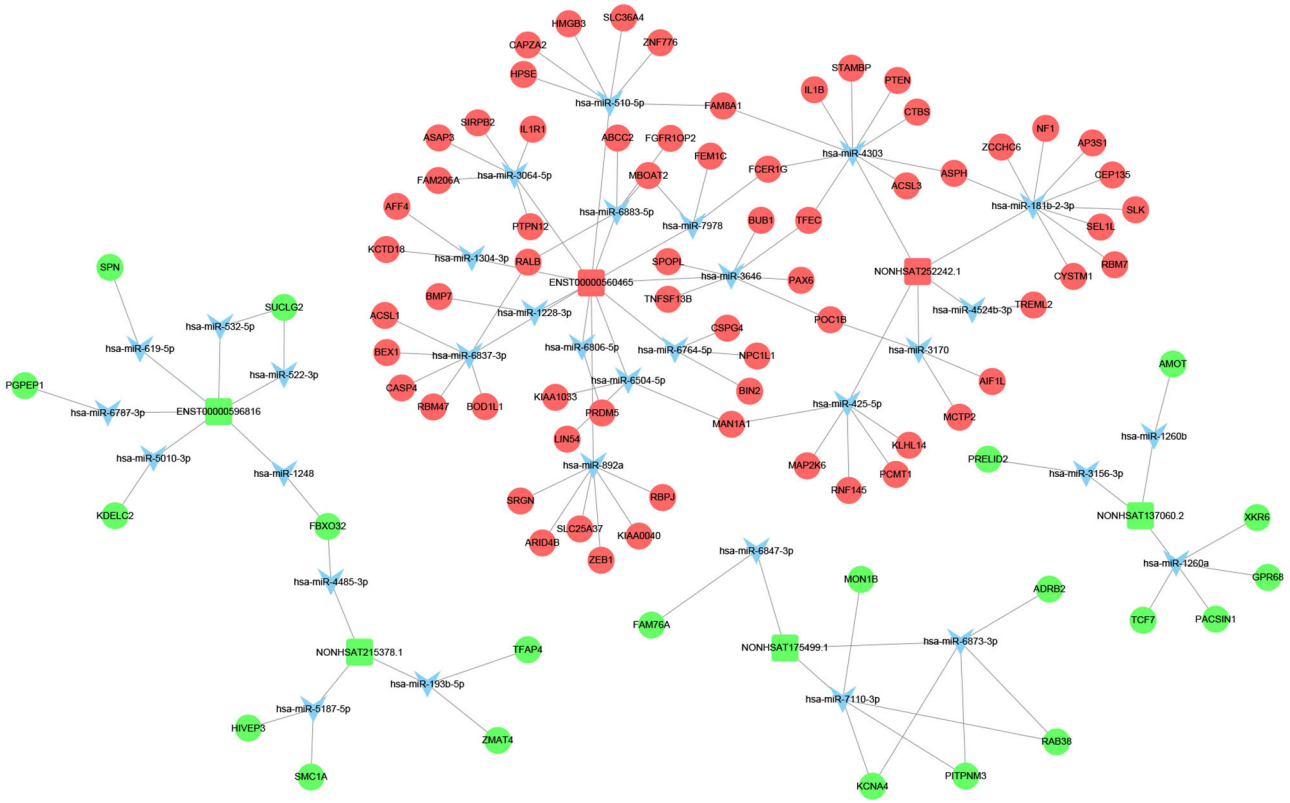

FIGURE 5 | LncRNA–miRNA–mRNA interaction network. Inverted triangles represent the predicted miRNAs; rectangle nodes represent lncRNAs; circles represent mRNAs (red, upregulated; blue, downregulated).

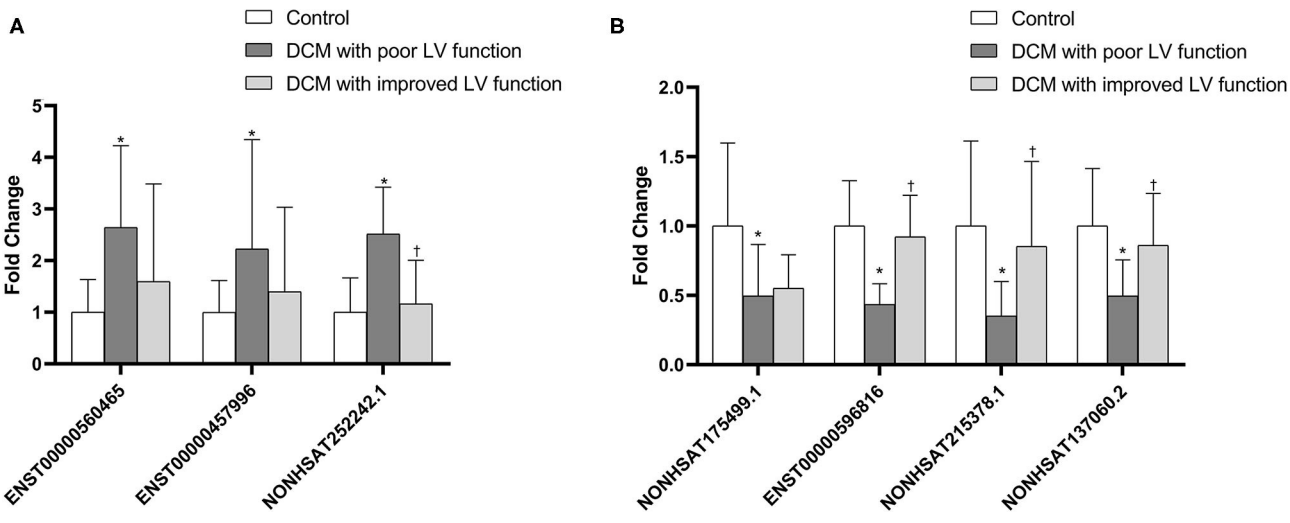

FIGURE 6 | Comparison of the relative lncRNA expression levels between DCM patients and normal children. The expression of 3 upregulated lncRNAs **(A)** and 4 downregulated lncRNAs **(B)** were validated using the $2^{-\Delta\Delta CT}$ method. $^{*}P < 0.05$ vs. control group. $^{\dagger}P < 0.05$ vs. DCM with poor LV function.

achieve the goal that regulates target genes expression. On the other hand, by using the co-expression network, we can analyze the gene regulation ability and obtain the core regulatory genes obtained by the samples analyzed in this experiment. Using this information, we constructed the lncRNA-mRNA co-expression network and lncRNA-miRNA-mRNA network. We

have identified 120 lncRNAs and 22 mRNAs altogether in the pathways mentioned above (Pearson's coefficient > 0.98). The lncRNA-mRNA network consisted of 142 net nodes and 149 connections of which each lncRNA was linked to 1–3 mRNAs, and each mRNA was linked to 1–18 lncRNAs (**Figure 4**). The five miRNAs with the highest binding potential for each lncRNA were predicted by using Cytoscape 3.5. The lncRNA-miRNA-mRNA network between the lncRNA related to the above pathways and the corresponding target mRNAs and target miRNAs was shown in **Figure 5**.

We screened out 9 lncRNAs based on the differentially expressed mRNAs and their different functions through the interaction network and the target gene prediction. The differentially expressed mRNAs involved in the above pathways include TLR3, PLIN4, PSEN1, BMP7, PPARG, etc. These 9 lncRNAs were selected for the next process.

Validation of lncRNA Expression

As mentioned earlier, we screened out 9 lncRNAs, of which 5 were up-regulated and 4 were down-regulated. After qRT-PCR analysis, these 9 lncRNAs were all amplified individually. However, among them, compared with the control group, the result of NONHSAT242978.1 and ENST00000637940 in the DCM group were opposite to the trends shown by the microarray data. Among the other 7 lncRNAs, 3 up-regulated (ENST00000560465, NONHSAT252242.1, ENST00000457996) and 4 down-regulated (NONHSAT175499.1, ENST00000596816, NONHSAT215378.1, NONHSAT137060.2) were consistent with the trend of microarray data results and are statistically significant ($P < 0.05$) (**Figure 6**). We also verified if these lncRNAs are differentially expressed in DCM patients with poor cardiac function and DCM patients with improved left ventricular function. The results show that only NONHSAT252242.1, ENST00000596816, NONHSAT215378.1, NONHSAT137060.2 had significant differences ($P < 0.05$, fold change > 2) in the group with improved left ventricular function as compared to the other DCM group.

DISCUSSION

DCM is the most common type of pediatric cardiomyopathy in the clinic, which is a major cause of heart failure, and often progresses to become severe over time. End-stage heart failure often requires heart transplantation, for which the prognosis is poor. To date, the etiology and pathogenesis of most cases of DCM have not been clearly determined. Current studies generally believe that the cause may be related to genetics or immunity. The genetic mechanism may account for 20–50% of DCM. Most of the inheritance is autosomal dominant inheritance, and there are also recessive, X-linked and mitochondrial inheritance (maternal inheritance) (23). There are currently more than 100 genes related to DCM (24), of which TTN is the most common accounting for 11% of DCM cases (25). Another popular hypothesis about related mechanisms is that DCM develops as a result of autoimmune myocarditis (26). These hypotheses provide a platform for us to study the pathogenesis of DCM.

According to the position of lncRNA in the genome relative to the protein-coding gene, it can be divided into seven categories: sense strand, antisense strand, intron lncRNA, bidirectional lncRNA, intergenic lncRNA, enhancer RNA, and circular RNA (27). More and more lncRNAs have established functional relevance in cardiovascular diseases (28), and their high tissue-specific expression indicates that in theory they can be used as disease markers or therapeutic targets. At present, the expression profile and functional mechanism of lncRNA in children with DCM are still unclear.

In this study, we first explored the expression profiles of lncRNA and mRNA of white blood cells extracted from peripheral blood collected from 3 DCM children and 3 normal children. As mentioned above, a total of 874 lncRNAs have expression differences ($P < 0.05$, fold change > 2), of which 370 are up-regulated and 504 are down-regulated. Then, in order to prove the accuracy of the microarray analysis, we selected 9 differentially expressed lncRNAs based on the functions of the respective differentially expressed mRNAs, which were verified by qRT-PCR in 25 children with DCM and 25 healthy children. Seven of them were confirmed to show the same upward or downward trend of IncRNAs in the DCM and healthy groups, including three that were up-regulated (ENST00000560465, NONHSAT252242.1, ENST00000457996) and four that were down-regulated (NONHSAT175499.1, ENST00000596816, NONHSAT215378.1, NONHSAT137060.2).

In addition, we wanted to know whether the expression levels of these 7 lncRNA molecules were different between the participants in the acute phase of DCM and the participants with relative improvement of the left ventricle after treatment. Therefore, we collected 20 peripheral blood samples from DCM children with improved left ventricular function, and verified whether there was a difference in expression of each lncRNA between the acute phase group and the improved group by qRT-PCR. The experimental results prove that three of the lncRNA (NONHSAT252242.1, ENST00000596816, NONHSAT215378.1, NONHSAT137060.2) are significantly different in the above two groups ($P < 0.05$, multiples > 2). This finding indicates that the expression of these molecules may be related to the severity of DCM, and may be used as a biomarker to measure the degree of recovery of DCM in the future.

The GO and KEGG analyses were used to determine the potential biological functions of differentially expressed IncRNAs and mRNAs. Among them, the most significant pathways related to DCM include the PPAR signaling pathway, Toll-like receptor signaling pathway, and Apoptosis pathways, which are related to inflammation, immunity, apoptosis and fibrosis, and serve as the basis for the 9 lncRNAs that we initially selected. Then, we constructed the co-expression network of lncRNA-mRNA and the lncRNA-miRNA-mRNA network in the signal pathway mentioned above to obtain the relationship between IncRNAs and mRNAs. These findings may enable us to further understand the pathogenesis of DCM.

In summary, the above experimental results indicate that the mutual regulation between lncRNA and mRNA may be involved

in the pathological process of DCM, and may be related to the improvement of left ventricular function which might suggest that these four molecules are more likely to become biomarkers related to DCM in children in the future.

It should be noted that there are some limitations in our study. Firstly, the sample size for microarray data analysis is small, and the false positive rate may be high. To account for this, we selected a larger number of samples for PCR validation, which helps to validate microarray data. Secondly, the part of the experiment here is mainly focused on bioinformatics analysis. The next step in future research experiments will be to select molecules according to the multiple to analyze for functional and mechanism verification.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) are: NCBI GEO SERIES (accession: GSE160986).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BH and DC designed the study. DC and WS performed all experiments described. BH and DJ supervised the study. JW and HJ analyzed the data. DC wrote the manuscript. BH and LZ approved the final version of the manuscript. All authors contributed to the article 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.2021.617298/full#supplementary-material

Supplementary Table 1 | Differentially expressed lncRNAs in pediatric dilated cardiomyopathy.

Supplementary Table 2 | mRNA profiles in pediatric dilated cardiomyopathy.

Supplementary Table 3 | *Cis*-target genes of differentially expressed lncRNAs.

Supplementary Table 4 | *Trans*-target genes of differentially expressed lncRNAs.

Supplementary Table 5 | The prediction of co-expression network.

Supplementary Table 6 | miRNA binding sites for circRNAs.

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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.

frontiers in Pediatrics

REVIEW
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Pediatric Malignant Arrhythmias Caused by Rare Homozygous Genetic Variants in *TRDN*: A Comprehensive Interpretation

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Aim: To perform a comprehensive phenotype-genotype correlation of all rare variants in Triadin leading to malignant arrhythmias in pediatrics.

Methods: Triadin knockout syndrome is a rare entity reported in pediatric population. This syndrome is caused by rare variants in the *TRDN* gene. Malignant ventricular arrhythmias and sudden cardiac death can be a primary manifestation of disease. Although pharmacological measures are effective, some patients require an implantable defibrillator due to high risk of arrhythmogenic episodes.

Main Results: Fourteen rare genetic alterations in *TRDN* have been reported to date. All of these potentially pathogenic alterations are located in a specific area of *TRDN*, highlighting this hot spot as an arrhythmogenic gene region.

Conclusions: Early recognition and comprehensive interpretation of alterations in Triadin are crucial to adopt preventive measures and avoid malignant arrhythmogenic episodes in pediatric population.

Keywords: sudden cardiac death, arrhythmias, pediatric, genetics, triadin

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INTRODUCTION

The *TRDN* gene (HGNC: 12261, ID: 10345) encodes an integral transmembrane protein of the junctional sarcoplasmic reticulum called triadin, divided in cytoplasmatic, transmembrane and luminal domains (**Figure 1**) (1). Triadin forms a complex with ryanodine, junctin, and calsequestrin to create the sarcoplasmic reticulum calcium release unit. Therefore, triadin is essential for normal function of both cardiac and skeletal muscle, as reported in knock-out mouse models (2–4).

In 2012, a homozygous alteration in *TRDN* was reported in association with a malignant arrhythmogenic phenotype (5). Three years later, the term "Triadin KnockOut Syndrome (TKOS)" was proposed as a syndrome leading to high risk of arrhythmias caused by homozygous *TRDN* alterations, mainly in infants and young populations (6). However, a recent study demonstrated that TKOS is a rare clinical entity that does not contribute meaningfully to either sudden infant death syndrome or sudden unexplained death in the young (7).

The International TKOS Registry highlighted a correlation between rare *TRDN* variants in homozygotes and aggressive arrhythmogenic phenotypes characterized by T-wave inversion in precordial leads, transient QT prolongation, and recurrent ventricular arrhythmias. Although few genetic alterations in heterozygotes have been reported, they show moderate arrhythmogenic phenotypes (8). To date, a limited number of pathogenic alterations have been reported in *TRDN* (**Figure 1**, **Table 1**). All alterations considered pathogenic have been associated with recurrent episodes of ventricular fibrillation (VF), sudden cardiac arrest, and highly malignant forms of catecholaminergic polymorphic ventricular tachycardia (CPVT) or long QT syndrome (LQTS), particularly at early ages. However, patients do not show typical phenotypes of CPVT or LQTS, suggesting an overlapping arrhythmogenic phenotype that is highly lethal. We have performed a comprehensive analysis of all pathogenic and likely pathogenic variants reported thus far in *TRDN*.

METHODS

We exhaustively reviewed the literature reporting *TRDN* and cardiac features up to August 2020. Data were collected from Human Genome Mutation Database (HGMD) (www.hgmd.org), ClinVar (www.ncbi.nlm.nih.gov/clinvar/intro), the National Center for Biotechnology Information SNP database (www.ncbi.nlm.nih.gov/SNP), Index Copernicus (https://www.indexcopernicus.com/index.php/en/), Google Scholar (scholar.google.es), Springer Link (link.springer.com), Science Direct (www.sciencedirect.com), Excerpta Medica Database (www.elsevier.com/solutions/embase-biomedical-research), and the IEEE Xplore Digital Library (ieeexplore.ieee.org/Xplore/home.jsp). Concerning *TRDN*, we consulted NCBI (https://www.ncbi.nlm.nih.gov/gene/?term=trdn), Genome Browser—Genomics Institute Sant Cruz, University of California (https://genome.ucsc.edu), GeneCards (https://www.genecards.org), and Genetics Home Reference (https://ghr.nlm.nih.gov). In addition, we obtained data for amino acid sequence or conservation among species (UniProt, www.uniprot.org) and protein–protein interactions (STRING, https://string-db.org).

Identified genetic variants were contrasted with variant data from Exome Variant Server (evs.gs.washington.edu/EVS) and Genome Aggregation Database (gnomad.broadinstitute.org, GnomAD), including recently added data concerning copy number variations. Genetic data were independently evaluated by three expert clinical geneticists and classified according to American College of Medical Genetics and Genomics (ACMG) guidelines (9). The PM2 item in the ACMG classification was considered fulfilled if minor allele frequency (MAF) in relevant population databases was $\leq$0.1% (10). For disease-causing variants, the majority of pathogenic variants were extremely rare in frequency (<0.001%) (11). PVS1 was only used for variants in genes with well-documented loss-of-function (www.ncbi.nlm.nih.gov/projects/dbvar/clingen) (12). Finally, all investigators discussed all data and agreed on final classification of all variants to avoid any bias.

RESULTS AND DISCUSSION

More than 20 years ago, triadin was stated as a key element maintaining regular heart rhythm via cardiac Ca^{2+} release, accompanied by its binding partners ryanodine-2, calsequestrin-2, and junctin (13, 14). However, the first association of alterations in *TRDN* as a cause of CPVT was not reported until 2012 (5). In this case, the authors reported two families showing a similar aggressive arrhythmogenic phenotype characterized by numerous polymorphic or bidirectional ventricular tachycardia. In the first family, from French West Indies, a homozygous deletion in exon 2 (c.del53_56, p.Asp18Alafs*14) was identified in a 2-year-old boy that presented with syncope followed by cardiac arrest at exercise. Resting electrocardiogram (ECG) following cardiac resuscitation showed numerous polymorphic or bidirectional ventricular extra beats and runs of polymorphic ventricular tachycardia. Our comprehensive analysis based on data currently available concluded a definite pathogenic role of this rare variant only in homozygous form (**Table 1**). In the second family, from Western France, two rare variants (c.176C>G, p.Thr59Arg/c.613C>T, p.Gln205*) were identified in a compound heterozygous form in a 26-year-old man with recurrent episodes of syncope during exercise since infancy. Exercise testing showed numerous bidirectional ventricular extra beats. Relatives carrying only one of these two variants did not show any clinical symptoms (5). Taking all data into account, we concluded that p.Thr59Arg may be highly deleterious in homozygous but not heterozygous form. In contrast, p.Gln205* seems to play a pathogenic role only in homozygosis (**Table 1**).

In 2015, Altmann et al. performed a comprehensive study in a cohort of 34 unrelated patients diagnosed with LQTS to identify the genetic cause of the disease (6). They identified five patients who showed similar aggressive arrhythmogenic phenotypes characterized by extensive T-wave inversion in precordial leads V1 through V4, with either persistent or transient QT prolongation or severe disease expression of exercise-induced cardiac arrest. Most patients were <10 years old and required aggressive therapy. Genetic analysis identified potential pathogenic rare *TRDN* variants in homozygous (c.del53_56/p.Asp18Alafs*14, and p.Lys147fs*) or compound heterozygous form (p.Lys147*, -c.438_442del-, and p.Asn9fs*5 -c.22+29A>G, IVS1dsA-G+29-). The p.Asp18Alafs*14 variant was identified in a young girl in homozygosis, and this

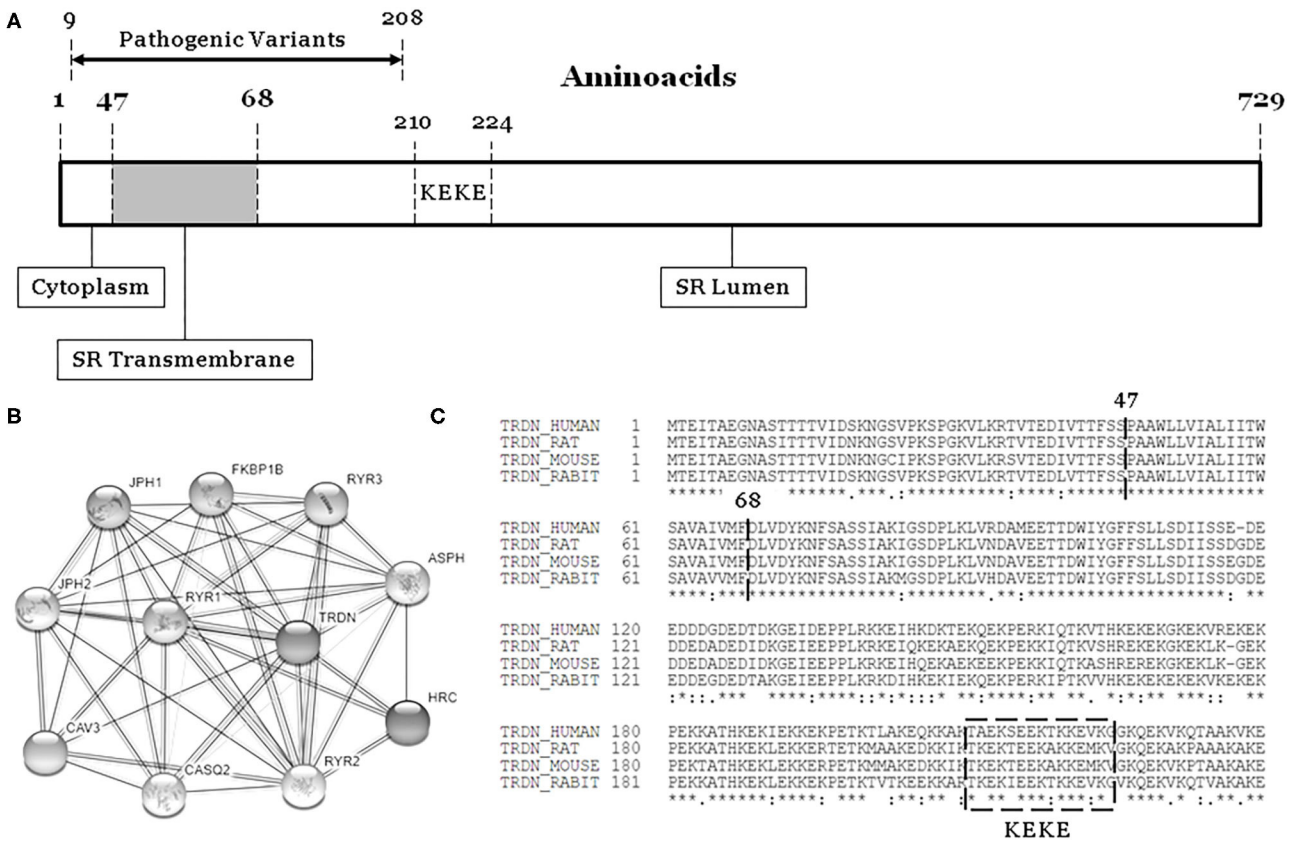

FIGURE 1 | The TRDN structure and closest interaction network. **(A)** Three domains: Cytoplasm, Transmembrane, and Lumen. **(B)** Network of 10 closest proteins to Triadin. **(C)** Conservation between species of main regions containing pathogenic variants (9–208). SR, sarcoplasmatic reticulum.

pathogenic rare variant had already been identified by Roux-Buisson et al. (5). Three unrelated patients carried the same homozygous frameshift deletion (c.438_442del). Parents with the same rare variant in heterozygosis did not show any symptoms. Our comprehensive analysis based on currently available data concluded a definite pathogenic role of p.Lys147* but only in homozygous form (**Table 1**). The last patient was an infant boy carrying p.Lys147*/c.438_442del- and c.22+29A>G in a compound heterozygous form (6). Our comprehensive genetic analysis concluded that both p.Asn9fs*5 and p.Lys147* variants should remain classified as ambiguous significance in heterozygosis. Also, Rooryck et al. published in 2015 a family from Western France in which two young sisters suffered aggressive CPVT episodes (15). Both sisters carried c.613C>T/p.Glu205* and c.22+29A>G in heterozygosis. These two heterozygous rare variants were inherited from different parents, fitting with an autosomal recessive mode of inheritance. Both parents were asymptomatic. Our comprehensive genetic analysis concluded ambiguity of p.Asn9fs*5 and p.Gln205* in heterozygosis according to previous published data (**Table 1**). However, a combination of both rare variants seems to play a deleterious role.

One year later, Walsh et al. (16) reported two 2-year-old siblings who showed aggressive CPVT phenotypes with recurrent episodes of VF despite β-blockade and internal cardiac defibrillator implantation. A novel compound heterozygous pathogenic complex in *TRDN* was identified: p.Asp18Alafs*13, previously reported as pathogenic in the homozygous state, and c.502G>T/p.Glu168*, reported as novel. Each heterozygous variant was inherited from a different parent, both of whom remained asymptomatic. Our comprehensive genetic analysis concluded an ambiguous role of p.Asp18Alafs*13 in the heterozygous state and a potential pathogenic role of p.Glu168* both in homozygous and heterozygous states (**Table 1**).

In 2018, the first and only homozygous deletion of *TRDN* (exon 2) was published (17). The patient was a 16-month-old infant who presented the most severe arrhythmogenic phenotype described thus far—it was characterized by recovered cardiac arrest, recurrent VF despite beta-blockade and flecainide, T-wave inversion in anterior precordial leads, and prolonged rate-corrected QT of 490 ms. Neither parent ever showed arrhythmogenic symptoms, and genetic analysis identified the same deletion in both parents but in heterozygosis. In addition, the index case also carried a missense variant in *KCNE2* (c.170T>C, p. Ile57Thr), which was previously described in LQTS but is currently classified as likely benign mainly due to high population frequency (MAF: 0.105%). Our comprehensive genetic analysis concluded a pathogenic role of homozygous deletion in exon 2 of *TRDN* (**Table 1**).

TABLE 1 | Genetic data of rare variants in *TRDN*.

Nucleotide change	Protein change	Zygosity	dbSNP	EVS MAF (%) (EA/AA/All)	gnomAD (MAF%)	ClinVar (disease)	HGMD (disease)	ACMG score homozygosis	ACMG score heterozygosis	TRDN domain
c.22+29A>G	IVS1dsA-G+29	Hetero	rs774068079	NA	1/247606 (0.0004%) Heterozygosis	NA	CS155261 (LQTS)	LP	VUS	C
c.53_56 delACAG	p.Asp18 Alafs*14	Hetero/ Homo	rs768049331	0.0/0.026/ 0.008 Heterozygosis	13/280480 (0.004%) Heterozygosis	P (CPVT)	CD124196 (CPVT)	P	VUS	C
Deletion Exon 2	-	Homo	NA	NA	NA	NA	CG1817756 (VF)	P	VUS	C/T
c.167T>C	p.Leu56Pro	Homo	NA	NA	NA	NA	NA	P	VUS	T
c.176C>G	p.Thr59Arg	Hetero	NA	NA	NA	NA	CM124195 (CPVT)	P	VUS	T
c.176C>T	p.Thr59Met	Hetero	rs397515459	NA	4/249154 (0.001%) Heterozygosis	VUS (CPVT)	CM193558 (CA)	LP	VUS	T
c.232+2T>A	IVS2dsT-A+2	Hetero	NA	NA	NA	NA	CS193557 (CA)	P	LP	L
c.423delA	p.Glu142 Lysfs*33	Homo	NA	0.218/0.122/ 0.188 Heterozygosis	NA	NA	CD193555 (CA)	P	LP	L
c.438_442 delTAAGA	p.Lys147*	Hetero/ Homo	rs970179891	NA	2/89152 (0.002%) Heterozygosis	NA	CD155260 (LQTS)	P	LB	L
c.484+1189G>A (c.485-24G>A)	-	Hetero	NA	NA	NA	NA	NA	P	P	L
c.502G>T	p.Glu168*	Hetero	NA	NA	NA	NA	CM160950 (CA)	P	LP	L
c.545dupA	p.Lys183Glufs*9	Homo	NA	NA	NA	NA	CI193559 (CA)	P	LP	L
c.613C>T	p.Gln205*	Hetero/ Homo	rs397515458	NA	5/163614 (0.003%) Heterozygosis	P (CPVT)	CM124194 (CPVT)	P	VUS	L
c.618delG	p.Ala208 Leufs*15	Homo	NA	NA	NA	NA	CD193556 (CA)	P	LP	L

ACMG, American College of Medical Genetics and Genomics; C, cytoplasmatic; CA, cardiac arrest; ClinVar, clinical variation; CPVT, catecholaminergic polymorphic ventricular tachycardia; DM, disease mutation; EVS, exome variant server (EA, European-American; AA, African-American; All, all populations); gnomAD, genome aggregation database; HGMD, human genome mutation database; L, luminal; LB, likely benign; LQTS, long QT syndrome; LP, likely pathogenic; MAF, minor allele frequency; NA, no data available; P, pathogenic; T, transmembrane; VF, ventricular fibrillation; VUS, variant of uncertain significance.

In 2019, the first International TKOS Registry was launched (8). Data from its initial 21 patients showed that TKOS is a potentially lethal syndrome, mainly at a young age, and is characterized by T-wave inversions in precordial leads, transient QT prolongation, and recurrent VF despite pharmacological treatment. Five new rare and potentially pathogenic variants were identified: three in homozygosis (c.423del/p.Glu142Lysfs*33, c.545dup/p.Lys183Glufs*9, and c.618del/p.Ala208Leufs*15), and two in compound heterozygosis (c.232+2T>A/IVS2dsT-A+2, and c.176C>T/p.Thr59Met). Our comprehensive genetic analysis concluded a pathogenic role of all these rare variants but only in homozygous form (**Table 1**). Both variants in heterozygosis were classified as likely pathogenic (IVS2dsT-A+2) or ambiguous significance (p.Thr59Met) alone, but a combination of both in heterozygosis should be considered deleterious. Further, a novel homozygous rare variant (c.167T>C, p.Leu56Pro) in *TRDN* has recently been reported (18). This 2-year old boy was resuscitated from sudden cardiac arrest and had frequent VF episodes despite beta-blocker plus flecainide therapy. He received an implantable cardiac

defibrillator (ICD). Both parents remained asymptomatic and carried the same rare variant but in heterozygosis. Functional studies reported in the same study support a pathogenic role for homozygous p.Leu56Pro. We definitely classify this rare variant in homozygosis as pathogenic (**Table 1**).

Recently, genome sequencing and *TRDN*-specific trio analysis were performed on a family (19). The index case was a 13-year-old boy who had his first cardiac arrest at the age of 18 months. He underwent placement of an ICD as well as left cardiac sympathetic denervation. Genetic analysis identified a maternally inherited c.22+29A>G variant, previously reported in 5 patients with TKOS from 3 unrelated families, leading to alternative splicing in the heterozygous form (6, 15). In addition, a novel deep intronic variant c.484+1189G>A (also annotated as c.485-24G>A) was identified in the index case. Functional studies determined that the last intronic variant not only disrupts proper splicing of exon 6a but also completely abolishes the CT1 transcript, ultimately leading to a *TRDN* null allele in the patient. Both parents had normal ECGs and negative personal and family histories of cardiac-related events. Taking all data into account, the variant c.22+29A>G in heterozygosis should be considered to have no conclusive deleterious role, while c.484+1189G>A should be classified as pathogenic (**Table 1**).

CONCLUSIONS

Our retrospective study concludes that homozygous/compound heterozygous rare variants in *TRDN* are associated with highly malignant arrhythmogenic phenotypes. Arrhythmias usually occur at young ages, and pharmacological treatment is mandatory. Aborted sudden cardiac arrest is not rare, and implantable cardiac defibrillator is recommended to prevent new episodes. Pathogenic alterations are located in the first 208 amino acids of the protein, suggesting a hot spot associated with aggressive arrhythmogenic syndromes. Early identification and comprehensive analysis of rare *TRDN* variants may help adopt preventive measures to reduce risk of lethal episodes.

AUTHOR CONTRIBUTIONS

OC, EA, JB, and RB developed the concept. OC, AF-F, SC, PJ, AG-Á, JC, EM, and GS-B acquired, pre-processed, and analyzed the data. OC, AF-F, SC, VF, and GS-B prepared the manuscript. OC and RB supervised the study. All authors contributed to manuscript revision, read and approved the current submitted version.

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frontiers in Pediatrics

REVIEW
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Hypertrophic Cardiomyopathy in Children: Pathophysiology, Diagnosis, and Treatment of Non-sarcomeric Causes

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Hypertrophic cardiomyopathy (HCM) is a myocardial disease characterized by left ventricular hypertrophy not solely explained by abnormal loading conditions. Despite its rare prevalence in pediatric age, HCM carries a relevant risk of mortality and morbidity in both infants and children. Pediatric HCM is a large heterogeneous group of disorders. Other than mutations in sarcomeric genes, which represent the most important cause of HCM in adults, childhood HCM includes a high prevalence of non-sarcomeric causes, including inherited errors of metabolism (i.e., glycogen storage diseases, lysosomal storage diseases, and fatty acid oxidation disorders), malformation syndromes, neuromuscular diseases, and mitochondrial disease, which globally represent up to 35% of children with HCM. The age of presentation and the underlying etiology significantly impact the prognosis of children with HCM. Moreover, in recent years, different targeted approaches for non-sarcomeric etiologies of HCM have emerged. Therefore, the etiological diagnosis is a fundamental step in designing specific management and therapy in these subjects. The present review aims to provide an overview of the non-sarcomeric causes of HCM in children, focusing on the pathophysiology, clinical features, diagnosis, and treatment of these rare disorders.

Keywords: hypertrophic cardiomyopathy, etiology, children, diagnosis, treatment

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a myocardial disease characterized by left ventricular (LV) hypertrophy not solely explained by abnormal loading conditions (1). HCM is a common genetic disorder in adults, with an estimated prevalence of 1:500 (1); on the contrary, it is rare in children but carries an important risk of morbidity and mortality (2). Compared with adult HCM, pediatric HCM is a more heterogeneous group of disorders. Other than mutations in sarcomeric genes, which represent the most important cause of HCM both in adults and in children (40–60% of cases) (1–7), the other causes of HCM include inherited errors of metabolism (i.e., glycogen storage diseases (GSDs), lysosomal storage diseases, and fatty acid oxidation disorders), neuromuscular diseases, malformation syndromes (i.e., RASopathies), and mitochondrial disease (**Table 1**), which

TABLE 1 | Causes of hypertrophic cardiomyopathy in children.

Sarcomeric	
Malformation syndromes: RASopathies	Noonan syndrome Noonan syndrome with multiple lentigines Costello syndrome Cardiofaciocutaneous syndrome
Glycogen storage diseases	Pompe disease (glycogen storage disease type IIa) Danon disease (glycogen storage disease type IIb) Cori–Forbes disease (glycogen storage disease type III) PRKAG2 syndrome
Lysosomal storage diseases	Mucopolysaccharidoses
Mitochondrial disorders	
Fatty acid oxidation disorders	Very long-chain acyl-CoA dehydrogenase deficiency Multiple-acyl-CoA dehydrogenase Long-chain hydroxyacyl-CoA dehydrogenase Carnitine-acylcarnitine translocase Carnitine palmitoyltransferase II Carnitine-acylcarnitine translocase deficiency
Endocrine disorders	Primary hyperinsulinism Infant of a mother with diabetes mellitus Acromegaly

globally represent up to 35% of children with HCM (2, 8). Sometimes, HCM is a feature of genetic syndromes associated with congenital heart disease (CHD), such as Noonan syndrome (NS), in which, for example, valvular pulmonary stenosis and HCM can be associated (3).

According to the 2020 American Heart Association/American College of Cardiology (AHA/ACC) guidelines, diagnosis of sarcomeric HCM in children requires an LV wall thickness more than 2 standard deviations from the predicted mean in children with a positive family history or a positive genetic test, and more than 2.5 in those without (9); however, specific *z* score thresholds have not been independently standardized.

The latest classification of cardiomyopathies in children of the AHA (10) has classified HCM in primary HCM, if a mutation in sarcomeric genes represents the cause of the disorder, and secondary HCM, if the disorder is associated with a non-sarcomeric cause; for the purpose of this document, we will refer to this classification.

In non-sarcomeric HCM, although the increased LV wall thickness can simulate that of sarcomeric form, the pathophysiology, the natural history, and the treatment are different. Thus, in subjects who meet the threshold for HCM diagnosis, causal predisposition or addition phenotypic characteristics need to be evaluated to identify the underlying cause (1, 3, 10, 11). To early identify the etiology of HCM, several diagnostic markers (obtained by pedigree analysis, physical examination, electrocardiography, echocardiography, and laboratory tests), the so-called "red flags," have been recommended to guide specialized diagnostic testing, including genetic analysis (1, 3, 10, 11) (**Figures 1**, **2**). The age of presentation can be considered a "red flag" for specific causes of HCM and also has a prognostic role. For example, HCM presenting before 1 year of age shows a worse prognosis (2) and is principally caused by inherited errors of metabolism or malformation syndrome (e.g., RASopathies) (**Figure 3**). On the contrary, mutations in sarcomeric genes represent the most important causes of HCM outside infancy, with a better prognosis compared with non-sarcomeric forms of HCM (2, 12, 13).

In recent years, several targeted approaches for specific etiologies of HCM have emerged (14, 15). Thus, the etiological diagnosis of HCM in children is a fundamental step in designing specific management and therapy in these subjects. The present review aims to provide an overview of the specific causes of HCM in children, focusing on the pathophysiology, diagnosis, and treatment of these rare disorders.

MALFORMATION SYNDROMES: RASOPATHIES

Introduction

RASopathies are a group of genetic disorders caused by mutations in RAS-MAPK cascade, which constitute, taken together, one of the largest groups of malformation syndromes, with a prevalence of 1:1,000–2,500 children (16–18). They include NS, NS with multiple lentigines (NSML; formerly known as LEOPARD syndrome), Costello syndrome (CS), cardiofaciocutaneous syndrome (CFC), neurofibromatosis type 1 (NF1), and Legius syndrome (LS).

Besides cardiac involvement, these diseases show a common clinical pattern whose major features are craniofacial dysmorphology, hypotonia, neurocognitive impairment, short stature, predisposition to various pediatric cancers, and cutaneous, muscular, and ocular abnormalities (19–21).

Clinical Presentation and Diagnostic Considerations

Noonan Syndrome

NS [Online Mendelian Inheritance in Man (OMIM) code #163950] is a developmental multisystemic disorder transmitted with an autosomal dominant pattern (17), and it is the second most common syndromic cause of CHD after trisomy 21 (22). Patients affected by NS are characterized by specific craniofacial features, including broad forehead, down slanting palpebral fissures, hypertelorism, and low-set ears; almost 10% of them have neurosensorial deafness or auditory deficits in the low-frequency range (17). Short stature is a common manifestation of the syndrome as the puberal grow spurt is usually attenuated (23). Up to 80% of male patients have unilateral or bilateral cryptorchidism (24). Among dermatological abnormalities, hypo- or hyperpigmentation can occur, including cafè-au-lait spots, keratosis pilaris, or multiple pigmented nevi (25). In most individuals, intelligence is within the normal range;

Possible Neonatologist Scenario:

Infant with LVH and Hypoglycemia

Possible causes:

Without Metabolic Acidosis

A. Hyperinsulinism

- Maternal diabetes
- Congenital hyperinsulinism (transient or persistent)
- *Insulin-resistance syndromes*

B. Disorders of fatty acid oxidation

With Metabolic Acidosis

A. Glycogen storage disorders

B. Mitochondrial disorders

Diagnostic algorithm:

1. Confirm hypoglycemia.

2. Consider checking urine organic acids, beta-hydroxybutyrate, insulin, free fatty acid, lactate.

3. Refer to third level cardiomyopathy centre to confirm the diagnosis and further management.

Possible Neonatologist or Pediatrician Scenario:

Infant or Children with - LVH and Dysmorphisms and/or Cutaneous Abnormalities

Possible causes:

A. RAS-MAPK disease

- *Noonan Syndrome*
- *NSML*
- *Costello Syndrome*
- *Cardio-facio-cutaneous Syndrome*

B. Other genetic syndrome

- LVH and Neuromuscular Symptoms

Possible causes:

With Ataxia

- *Friedrich Ataxia*

With hypotonia, muscle weakness and/or CPK elevations

- *Glycogen storage disorders*
- *Mitochondrial disorders*

Refer to third level cardiomyopathy centre to confirm the diagnosis and further management.

Possible Pediatrician or Pediatric Cardiologist Scenario:

Infant or Children with LVH and other ECG or Echo "Red Flags" (see Figure 2)

Possible causes:

A. Malformation Syndromes

B. Metabolic Syndromes

C. Mitochondrial Diseases

D. Neuromuscular Syndromes

E. Endocrine Disorders

F. Others

Refer to third level cardiomyopathy centre to confirm the diagnosis and further management.

FIGURE 1 | Possible scenarios in clinical practice. LVH, left ventricular hypertrophy; NSML, Noonan syndrome with multiple lentigines.

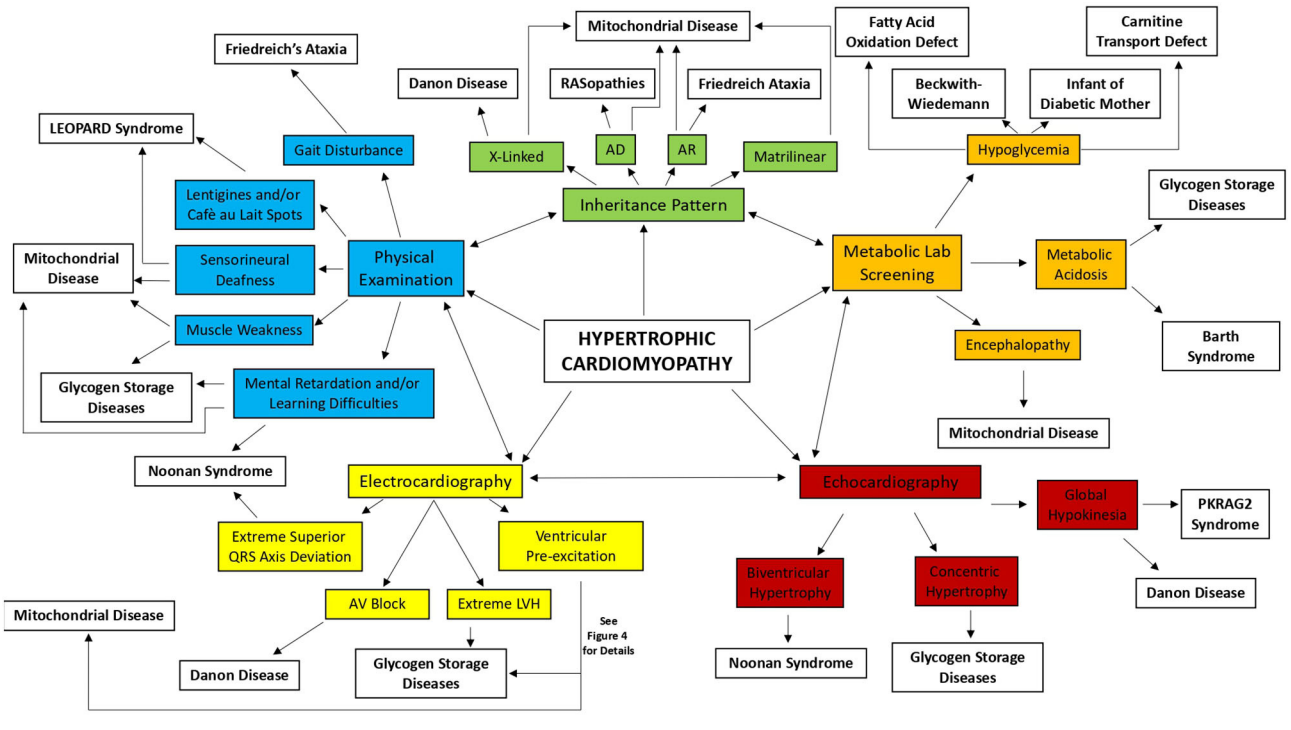

FIGURE 2 | Diagnostic approach for the infant and young child with hypertrophic cardiomyopathy. AD, autosomal dominant; AR, autosomal recessive; AV, atrioventricular; LVH, left ventricular hypertrophy.

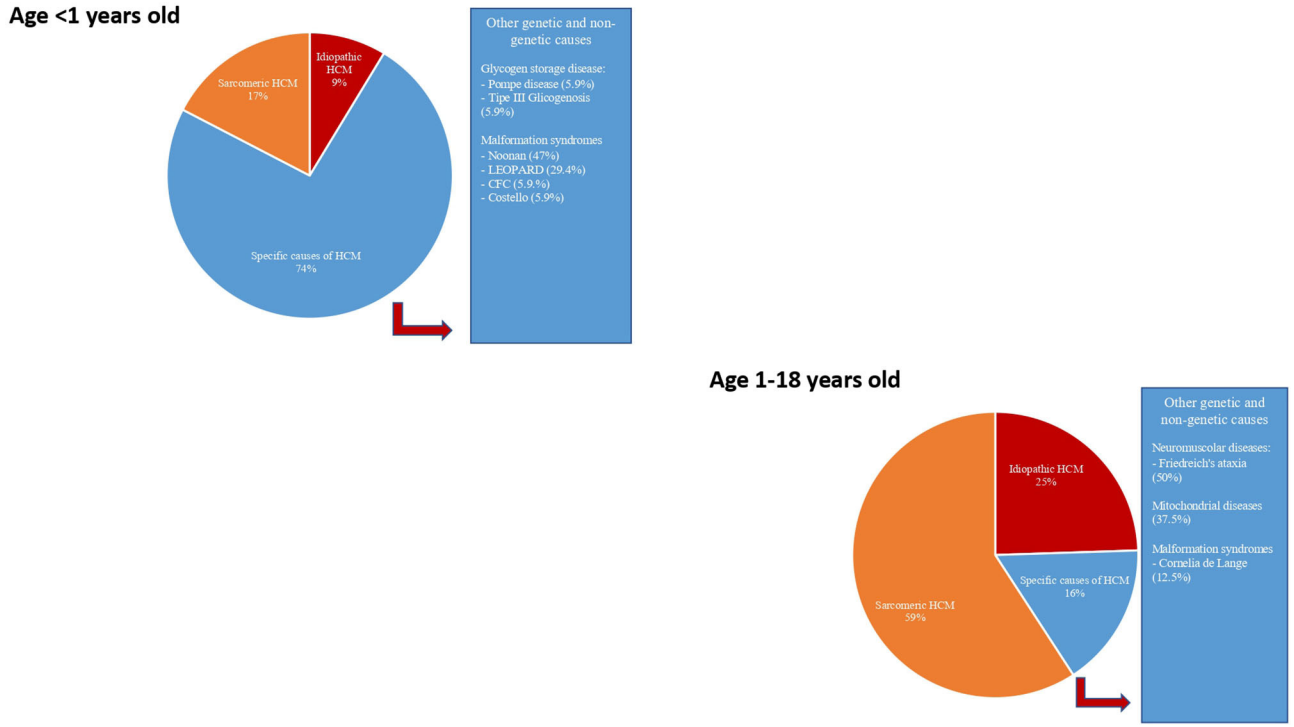

FIGURE 3 | Etiology of hypertrophic cardiomyopathy in infants (age < 1 year) and in a young child (age 1–18 years). Modified from Limongelli et al. *Int J Cardiol* 2020 (3).

nonetheless, Roelofs et al. showed that almost 60% of the individuals have a significant difference between verbal and non-verbal intelligence quotient (26). Several malignancies have been reported in patients with NS, particularly juvenile myelomonocytic leukemia, acute myelogenous leukemia, and embryonal rhabdomyosarcoma (27).

The first NS pathogenetic gene was described in 2001 by Tartaglia et al. and identified as the tyrosine phosphatase *PTPN11*, whose missense loss-of-function mutations cause overactivation of the RAS-MAPK cascade (28). Subsequent studies showed that *PTPN11* accounts for almost 50% of the cases (29) (**Table 2**). *SOS1* mutations represent the second most common cause of NS, accounting for almost 15% of the patients (30). A rare subset of NS patients who show a particular phenotype of loose anagen hair (also known with the eponym of Mazzanti syndrome) is caused by a single mutation is *SHOC2*, a scaffold protein linking Ras to *RAF1* (31). Later studies identified other genes underlying NS, namely, *KRAS* (32), *RAF1* (33), *MAP2K1* (34), *BRAF* (35), *NRAS* (36), and *CBL* (37).

Noonan Syndrome With Multiple Lentigines

Among RASopathies, NSML, also known as LEOPARD syndrome (OMIM code #151100), is similar to NS, but it is characterized by multiple lentigines, sensorineural deafness, ocular hypertelorism, abnormal genitalia, and a higher prevalence of HCM and conduction abnormalities (38). Genetic analysis showed that NSML, similar to NS, is caused by different mutations in *PTPN11* (39). Rarely, mutations in *RAF1* and *BRAF*, which are more common in CFC, have been identified in NSML (39, 40).

Costello Syndrome

CS (OMIM code #218040) is a genetic syndrome with many overlapping features with NS (19). Differential diagnosis with NS is seldom feasible during infancy and becomes possible only in adult life (41). Both syndromes can present HCM at very infantile onset, but infants with CS have a lesser prevalence of pulmonary stenosis or complex CHD (40–42). Phenotypic features may be apparent in intrauterine life, with polyhydramnios *in utero*, prematurity, and increased birth weight. Children with CS have coarse facial features, with a prominent forehead, epicanthal folds, and depressed nasal bridge. The skin is soft and redundant with palmar and plantar creases. Almost 72% of CS patients show evidence of cutaneous papilloma, localized especially at the nose. These neoplasms are not common in other RASopathies and could offer a clue for differential diagnosis (19, 42). CS is caused by heterozygous activating mutations in *HRAS*. More than 80% of CS patients have a p.G12S substitution, which causes reduction of the GTPase activity of HRAS, with subsequent overactivation of the MAPK pathway (43).

Cardiofaciocutaneous Syndrome

Among the rarest RASopathies, CFC (OMIM code #115150) shows many overlapping features with NS and CS, including

TABLE 2 | Clinical manifestations, mutated genes, and classical heart defects with their relative prevalence among different specific causes of pediatric HCM.

Specific cause	Gene	Clinical features
RASopathies		
Noonan syndrome	*PTPN11* *SOS1* *RAF1* *RIT1* *SHOC2* *NRAS* *CBL*	PVS (65%), HCM (20–23%), atrioventricular septal defects (10–18%), ASD (10%), VSD (8.5%), PDA (6%), mitral valve abnormalities (2.5%), tetralogy of Fallot (<1%), aortic coarctation (<1%). PVS (70%), HCM (18%), ASD (14.5%), mitral valve abnormalities (3.6%). HCM (65%), PVS (21%), ASD (19.6%), mitral valve abnormalities (13%), tetralogy of Fallot (2.8%), aortic coarctation (1.8%). PVS (74%), HCM (36%), ASD (30%), PDA (7.4%), mitral valve abnormalities (13%), biventricular obstruction (<1%). PVS (32%), ASD (32%), HCM (29.7%), mitral valve abnormalities (27%), VSD (12.7%). HCM (39.3%), PVS (14.3%). PVS (50%), HCM (10%), ASD (10%), mitral valve abnormalities (10%).
Noonan syndrome with multiple lentigines	*PTPN11* *RAF1* *BRAF*	HCM (60–85%), biventricular hypertrophy (46%), LVOTO (40%), ventricular tachycardia, conduction abnormalities, mitral valve abnormalities (26–42%), PVS (21%), ASD (6%), atrioventricular septal defects, coronary artery abnormalities. HCM (100%), mitral valve abnormalities (100%), PVS (40%), high risk of SCD. HCM (60–85%).
Costello syndrome	*HRAS*	HCM (65%), atrial tachycardia (56%), PVS.
Cardiofaciocutaneous syndrome	*BRAF1* *MAP2K1–MAP2K2:* *KRAS*	PVS (45%), HCM (40%), ASD (30%), VSD (7.6%). tetralogy of Fallot (7%). PVS (65–100%), HCM (15–40%), septal defects (15%). PVS (40%), mitral valve abnormalities (26%), HCM (20%), ASD (20%), VSD (13%).
Glycogen storage disorders		
Pompe disease	*GAA*	LVH, short PR interval, hypertension, idiopathic stroke, cerebral artery aneurysms.
Danon disease	*LAMP2*	Short PR interval (75%), severe concentric LVH, conduction abnormalities.
Cori–Forbes disease	*AGL*	Concentric LVH (40%), biventricular hypertrophy (15%), HF, isolated septal hypertrophy.
PRKAG2 syndrome	*PRKAG2*	LVH, AV block (45–50%), SVT (38%), sick sinus disease (50%), SCD (10%), syncope.
Lysosomal storage disorders		
Hurler syndrome (MPS type 1)	*IDUA*	LVH, thickening of valve leaflets or papillary muscles, shortening of subvalvular apparatus, systemic hypertension, dilated cardiomyopathy with reduced EF, pulmonary hypertension, coronary artery disease, AV block.
Hunter syndrome (MPS type 2)	*IDS*	LVH, thickening of valve leaflets or papillary muscles, shortening of subvalvular apparatus, systemic hypertension, dilated cardiomyopathy with reduced EF, pulmonary hypertension, coronary artery disease, AV block.
Maroteaux–Lamy syndrome (MPS type 6)	*ARSB*	LVH, thickening of valve leaflets or papillary muscles, shortening of subvalvular apparatus, systemic hypertension, dilated cardiomyopathy with reduced EF, pulmonary hypertension, coronary artery disease, AV block.
Mitochondrial disorders		
MELAS syndrome	Mitochondrial genes	HCM (75%), dilated cardiomyopathy (12.5%), pulmonary hypertension (12.5%), stroke-like episodes.
MERRF syndrome	Mitochondrial genes	WPW (45–22%), dilated cardiomyopathy (22%), HF (11%).
CoQ10 deficiency	*COQ4*	HCM, bradycardia, HF.
Barth syndrome	*TAX*	Left ventricular non compaction, dilated cardiomyopathy, HCM, endocardial fibroelastosis, ventricular arrhythmias, SCD.
Friedrich's ataxia	*FTX*	HCM (75%), dilated cardiomyopathy, granular sparkling (rare), bundle branch block (15%).

ASD, atrial septal defect; HCM, hypertrophic cardiomyopathy; HF, heart failure; LVH, left ventricular hypertrophy; LVOTO, left ventricular outflow tract obstruction; PDA, patent ductus arteriosus; PVS, pulmonary valve stenosis; SCD, sudden cardiac death; VSD, ventricular septal defect; WPW, Wolff–Parkinson–White syndrome.

the typical facies, neurocognitive delay, hypotony, and learning disability (20). Differential diagnosis includes the presence of curly hair with sparse eyebrows and eyelashes (44), and neurological and ocular abnormalities like strabismus and nystagmus (45). Unlike CS, the genetic substrate of CFC is heterogeneous, with mutations in *BRAF1* (34), *MAP2K1*, *MAP2K2* (46), and *KRAS* accounting for 80% of the cases.

Neurofibromatosis Type 1 and Legius Syndrome

RASopathies also include NF1 (OMIM code #162200) and LS (OMIM code #611431), caused, respectively, by inactivating mutations of *NF1*, which encode for a GTPase activating protein, and SPRED1, which is a negative mediator of RAS-mediated RAF 1 activation. However, HCM is not commonly associated with NF1 and LS.

Hypertrophic Cardiomyopathy in RASopathies

With the exclusion of CHD, HCM is the most common cardiovascular abnormality observed in RASopathies (47, 48). HCM in RASopathies is characterized by a higher grade of ventricular hypertrophy, an increased prevalence, and a more severe pattern of LV outflow tract obstruction (LVOTO) than non-syndromic forms (49). Biventricular involvement is often described, because of the high prevalence of right ventricular hypertrophy (49). Obstructive forms of HCM are not solely explained by the systolic anterior motion of the mitral valve (MV), but other factors, such as accessory fibrous connective tissue causing subaortic stenosis, displacement of the papillary muscles, and anomalous insertion of the MV, have been described (47). The length of MV leaflets and chordal anatomic relationships are different compared with MV in non-syndromic subjects with HCM (50). To date, MV anomalies are a marker of complexity in HCM and have been associated with a high risk of reintervention and death (51). Myocardial ischemia is a common finding in HCM, reflecting the imbalance between oxygen supply and demand, and it is a major clinical issue in young adolescents with RASopathies (47). Coronary arteries abnormalities have been described in up to 30% of patients affected by RASopathies, and they could contribute to myocardial ischemia (52).

Moreover, cardiac arrhythmias are a major determinant of clinical prognosis in children affected by RASopathies and HCM (42). Supraventricular and ventricular ectopy have been described, and in severe cases, the occurrence of sustained ventricular tachycardia has been reported.

The initial evaluation of a patient affected by HCM should include systematic research for clinical clues or "red flags," obtained by pedigree analysis, clinical examination, ECG, imaging, and biochemical tests. In particular, the diagnosis of RASopathy may be suggested by facial dysmorphism, lentigines, sensorineural deafness, pulmonary stenosis, or biventricular hypertrophy (3, 53).

Hypertrophic Cardiomyopathy in Noonan Syndrome With Multiple Lentigines

HCM is diagnosed in almost 80% of patients affected by NSML, and it is associated with early-onset presentation and worse clinical outcome, with clinical evidence of LVOTO in up to 40% of the cases (38, 47, 54). Although HCM can be congenital in NSML, it is mostly observed in second infancy, where it precedes the appearance of multiple lentigines. Biventricular hypertrophy can be found in 46% of patients affected by NSML, in association with typical electrocardiographic findings, as a superiorly oriented mean QRS axis, q waves, prolonged QTc, and/or repolarization abnormalities (38). Clinically relevant genotype–phenotype correlations have been described: patients without PTPN11 mutations showed a higher prevalence of family history of sudden death ($p = 0.007$) and non-sustained ventricular tachycardias ($p = 0.05$). Of note, mutations of the exon 13 of the PTPN11 gene were associated with severe obstructive HCM (48).

Other cardiac manifestations in NSML include pulmonary valve stenosis, found in almost 23% of the affected individuals, and MV prolapse, clefting, or other morphological abnormalities, which are present in up to 42% of patients. Rarely, NSML has been associated with atrioventricular septal defects, LV non-compaction, and coronary arteries abnormalities (21).

Hypertrophic Cardiomyopathy in Costello Syndrome

Approximately 65% of patients affected by CS have HCM, and 40% of them show evidence of CHD (42). Most patients have subaortic septal hypertrophy; however, other patterns such as concentric LV hypertrophy, mild septal thickening, or biventricular involvement have been described (42). In a cohort of 126 patients affected by CS, the mean age at diagnosis was 4.6 years, and no fetal diagnosis of HCM was made. The clinical course was progressive in 38% of the patients and stable in 30% of the patients, and interestingly, almost 11% of the patients showed regression of cardiac hypertrophy.

Although atrial arrhythmias are common among patients with RASopathies, their prevalence seems to be higher in CS, where they can be diagnosed in up to 56% of the patients. Thus, the identification of focal atrial tachycardia could represent a diagnostic clue and, in combination with specific clinical features, should trigger the suspicion of an underlying CS (19, 32, 43). On the other hand, in patients with other RASopathies, multifocal atrial tachycardia (MAT) seems to be associated specifically with RAF1 mutations (42).

Hypertrophic Cardiomyopathy in Cardiofaciocutaneous Syndrome

HCM is diagnosed in almost 40% of individuals affected by CFC, although the most common cardiac abnormality is pulmonary valve stenosis, diagnosed in almost 45% of the patients (20). There are lacking data to compare HCM in CS to NS and CFC, although case reports suggest that severe subaortic obstruction requiring surgery is more frequent in CS than in NS and CFC (55).

Hypertrophic Cardiomyopathy in Noonan Syndrome

A lower prevalence of HCM has been described for NS: although more than 80% of the patients show cardiac abnormalities, the prevalence of HCM in NS has been estimated to be 20–23% (17). Prevalence of HCM is slightly higher in a subset of Noonan patients carrying the variant p.Ser2Gly in SHOC2 gene, also known as NS with loose anagen hair (31). NS-associated HCM occurs early in childhood, with a median age at diagnosis of 6 months (56). The distribution of LV hypertrophy is similar to idiopathic variants. Asymmetric septal hypertrophy is the most common pattern, described in 75.6% of Noonan patients. Noteworthy, apical cardiomyopathy is seldom described in NS (57). Electrocardiograms may show several abnormalities, also in the absence of structural abnormalities (56). The magnitude of LV hypertrophy was significantly higher in those with NS or NSML than non-syndromic forms [8.9 as opposed to 6.4

($p = 0.03$)]. Significant obstruction of LVOT is more common in NS or NSML than in idiopathic HCM (53 vs. 15%, $p = 0.02$). Dilatation of the coronary arteries has been described in 29% of the affected patients (49). Patients affected by NS and HCM also seemed to present a higher risk of dilated cardiomyopathy in adult life (21). The prognosis of the patients affected by NS and HCM is influenced by the high prevalence of pulmonary valve stenosis (described in almost 65% of the patients), and the coexistence of complex CHD as atrioventricular septal defects (10%), atrial septal defect (10%), and rarely tetralogy of Fallot, patent ductus arteriosus, and left-sided obstructions resulting in MV abnormalities (17, 21). Vascular abnormalities are also described, mainly aortic dissection, aortic root dilatation, and aneurysms of the sinuses of Valsalva (17).

Genotype–Phenotype Correlation in RASopathies: The Key to a Target Therapy?

Many clinically relevant genotype–phenotype correlations have been described in RASopathies (**Table 2**). Among patients with NS, pulmonary stenosis was more common in *PTPN11* and *SOS1* mutation patients, whereas HCM without pulmonary stenosis was more prevalent in carriers of *RAF1* mutations, where the prevalence of HCM is up to 65% (16, 40, 58). It has been suggested that HCM in RASopathies could be caused by increased activation through the RAS-MAPK cascade, causing cardiomyocyte hypertrophy and myocardial fiber disarray (21). Consequently, treatment with Mek1 inhibitors seemed to rescue the cardiac phenotype in mouse Raf1-mutated models (59). Trametinib, a highly selective Mek1/2 inhibitor that seemed to reverse hypertrophic phenotype within 4 months after initiation of treatment, is preceded by a favorable clinical response in a single patient with *RIT1*-associated HCM (60). On the other hand, specific missense mutations of *PTPN11* associated with NSML seem to cause increased activity through the mTOR–PI3K–AKT signaling pathway. Treatment of the *PTPN11* (Y279C) mice with rapamycin, an inhibitor of mTOR–PI3K–AKT pathway, normalized HCM (61). Recently, everolimus has been used to prevent acute decompensation of heart failure in severe HCM in patients with NSML, even though no reversal of HCM was observed (62) (**Table 3**).

Prognosis and Risk of Sudden Cardiac Death

In the subgroup of patients with RASopathies, HCM is a major determinant of the clinical prognosis. In a cohort study, children with NS presented a higher prevalence of congestive heart failure (CHF) (24 vs. 9%) compared with those with idiopathic HCM, and a significant early mortality rate (22% at 1 year) (64). Low cardiac output, significant diastolic dysfunction, and a higher number of interventions have been reported in patients who died for cardiac causes (51, 64).

The stratification of risk for sickle cell disease (SCD) among patients with RASopathies and HCM is not completely understood, and data have been extrapolated from larger studies including pediatric patients affected by sarcomeric HCM (65–67). Recently, an international multicentric observational cohort study including 572 children with HCM has validated age at diagnosis, history of recent unexplained syncope within 6 months before the diagnosis, documented non-sustained VT (defined as $\geq$3 beats at $\geq$120 bpm on ambulatory ECG), interventricular septal diameter (IVSD) z score, LV posterior wall diameter (LVPWD) z score, left atrial (LA) diameter z score, and peak resting LVOT gradient on echocardiography as risk factors for SCD in pediatric HCM. Nevertheless, patients with RASopathies were excluded from the analysis (68).

Specific etiologies, as the diagnosis of NSML and, in particular, an arrhythmogenic phenotype as the absence of PTPN11 mutation in NSML, might provide additional risk, but the available evidence is not conclusive to provide a prognostic stratification for SCD in children with RASopathies (48).

Treatment

General management of HCM in RASopathies is based on current clinical practice guidelines. Medical therapy, mainly based on beta-blockers, can be used to relieve symptoms and the degree of obstruction (1).

Surgical myectomy is feasible in NS and should be considered in patients who remain symptomatic despite maximal medical therapy (69). Orthotopic heart transplantation is rarely performed, and long-term follow-up studies are needed to assess prognostic implications of heart transplantation in these patients (70). However, it may be considered in those with advanced heart failure, or intractable ventricular arrhythmias, or severe diastolic dysfunction.

GLYCOGEN STORAGE DISEASES

GSDs represent an important cause of HCM in children and are characterized by the formation of glycogen-filled vacuoles in cardiomyocytes (71). Different GSDs are known to be associated with HCM (1, 72, 73); in particular, the most important are represented by Pompe disease (or GSD type IIa), Danon disease (or GSD type IIb), Cori–Forbes disease (or GSD type III), and PRKAG2 syndrome.

Pompe Disease (Glycogen Storage Disease Type IIa)

Introduction

Pompe disease (OMIM code #232300) is a rare and progressive metabolic disorder caused by the partial or complete deficiency of the acid alpha-glucosidase enzyme (GAA). This condition leads to a pathological accumulation of glycogen in several organs, in particular in the nervous system and muscles, including the heart (74).

It is inherited with an autosomal recessive pattern and is the result of homozygotic mutations in *GAA* (75), which encodes for an enzyme that is responsible for lysosomal glycogen hydrolyzation into glucose. The glycogen accumulation and the lysosomal membrane rupture in the muscle tissue lead to a massive leakage of lytic enzymes responsible for muscle damage (76, 77).

TABLE 3 | Advantages and disadvantages of the available treatments for Pompe disease.

HCM specific cause	Specific therapies and future perspective
RASopathies	*No specific treatment currently available* *MEK inhibitors (future perspective)* In mouse models of Noonan syndrome, the administration of MEK inhibitors during 4–10 weeks of life could prevent the development of cardiomyopathy. Data from a clinical report showed that MEK inhibitors can induce the regression of cardiac hypertrophy within 4 months of treatment. *mTOR inhibitors (future perspective)* In mouse models with PTPN11 mutation Y279C, the administration of rapamycin, an inhibitor of mTOR–PI3K–AKT pathway, induced regression of HCM.
Pompe disease	*Enzyme replacement therapy* Advantages: reduction of cardiac mass and reverse remodeling; long-term efficacy achieved in a subgroup of patients. Disadvantages: high doses required; highly dependent on level of M6PR; does not cross the BBB; possible adverse reactions: ventricular ectopy; transient fall of the left ventricular ejection fraction; immune-mediated reaction. *Gene therapy (future perspective)* Advantages: improved clearance of glycogen in the muscles; enhanced respiratory and cardiac performance; potential for expressing GAA directly in target tissues. Disadvantages: high vector doses needed; transgene immunogenicity
Danon disease	*No specific treatment currently available* *Gene therapy (future perspective)* In mouse models of Danon disease, LAMP2B gene transfer improves metabolic and physiologic function.
PRKAG2 syndrome	*No specific treatment currently available*
Cori–Forbes disease	*No specific treatment currently available* *Gene therapy (future perspective)* *In mouse models of Cori–Forbes disease, GDE gene transfer blocked glycogen accumulation in cardiac and skeletal muscles and improved the muscle functions.*
Mucopolysaccharidoses	*Enzyme replacement therapy* Advantages: effective on visceral organs; easy to administer. Disadvantages: has limited impact on poorly vascularized tissues; does not cross the BBB; requires continuous administration; can cause IgG antidrug antibodies formation; can cause infusion-related reactions. *Hematopoietic stem cell transplantation* Advantages: it is a permanent therapy; increases the long-term survival; can cause the interruption of the disease progression; can cross the BBB. Disadvantages: does not prevent valve dysfunction; can cause GVHD; can cause graft rejection. *Gene therapy (future perspective)* Advantages: it is a permanent therapy; potentially able to cross the BBB. Disadvantages: can cause off-target gene effects; the long-term effects are unknown. *Chaperone therapy (future perspective)* Advantages: can cross the BBB; it is not immunogenic. Disadvantages: can cause off-target adverse effects; it is not approved for all the MPSs type.
Mitochondrial disorders	*No specific treatment currently available, except for:* *CoQ_{10} deficiency:* CoQ_{10} supplementation. *Thiamine-responsive disorders:* thiamine supplementation. In mouse models of Barth syndrome, gene therapy rescued neonatal demise, prevented cardiac dysfunction, and reversed established heart disease. Several treatments are currently on investigations, such as MitoQ antioxidant, MTP-131 peptide (which reduce the ROS release and improving ATP synthesis), inhibitors of mPTP, mitochondrial transplantation, etc. (63)

BBB, brain–blood barrier; ERT, enzyme replacement therapy; GVHD, graft vs. host disease; M6PR, mannose-6-phosphate receptor; MPSs, mucopolysaccharidoses.

Clinical Presentation and Diagnostic Consideration

Pompe disease is classified in infantile form and late onset, when it presents before and after the 1st year of life, respectively.

The infantile form is generally classified into a classic Pompe disease, which generally presents with a rapidly progressive course with severe cardiomegaly, hepatomegaly, weakness, hypotonia, respiratory distress, infections, and feeding difficulties; and a non-classic Pompe disease, which represents a variant form with slower disease progression and mild or absent cardiomyopathy (78–80). Cardiac involvement of the infantile form of Pompe disease represents the dominant feature of the disease and the major determinant of mortality. Thus, in the infantile form of Pompe disease, chest radiography often shows cardiomegaly, while ECG shows a short PR interval and hypertrophy signs (76). Echocardiography often shows LV hypertrophy with or without LVOTO, mimicking HCM. In particular, cardiac involvement presents in the 1st months of life with severe ventricular hypertrophy (ranging from +2 to +10 or higher *z* score for LV mass), most notably in the posterior wall and the interventricular septum, which can appear also in intra-uterine life. The LV ejection fraction, initially preserved, tend to

fall in the first 5 months moving toward dilated cardiomyopathy with end-stage heart failure in the 1st year of age (81).

The late-onset form includes childhood, juvenile, and adult variants characterized by a slow course and a predominant skeletal muscle involvement (76). Other features of the late-onset form are represented by hypertension, due to loss of aortic compliance, and idiopathic stroke. Preexcitation syndrome, LV hypertrophy, and ascending aorta dilation represent possible cardiac features of late-onset Pompe disease. Respiratory failure is a possible lethal complication caused by the involvement of the respiratory muscle (82).

The diagnosis of Pompe disease is challenging given its heterogeneous presentation. Dry blood spot test is a valid screening method for patients with suspicion of Pompe disease, but the gold standard diagnostic test is the measurement of GAA enzyme activity in skin fibroblasts (74, 76). Indeed, the diagnosis is generally performed demonstrating a significant reduction in GAA enzyme activity and identifying the disease-causing mutations of the GAA gene.

Prognosis and Risk of Sudden Cardiac Death

As stated before, cardiovascular involvement is the major determinant of prognosis in patients with the infantile form of Pompe disease, with the possible evolution to refractory heart failure and death in the 1st year of age (76, 81). On the contrary, in the late-onset form, the prognosis is mainly dependent upon the age of onset with a slower disease progression in those patients manifesting later the disease. In this subgroup, the prognosis is dependent upon the extent of respiratory muscle involvement (82). Sudden cardiac death is extremely rare in patients with Pompe disease.

Treatment

Actually, the standard of care in patients with Pompe disease is represented by enzyme replacement therapy (ERT), based on the discovery of receptor-mediated uptake of lysosomal enzymes related to mannose-6-phosphate receptor (M6PR) (**Table 3**). The main goal of ERT is to slow down, stabilize, and reverse disease progression. Among the benefits of ERT, there is the reduction of cardiac mass, reverse remodeling, and improvement of cardiac function. The efficacy of this treatment has led to a reduction in the use of inotropes, diuretics, and vasodilators which can exacerbate the LVOTO (76). Recombinant GAA (rhGAA) doses used in patients with Pompe disease are markedly high compared with those used in other lysosomal storage diseases, probably because of the elevated liver absorption (more than 85%), limiting the muscle uptake. Possible side effects are rare and include a transient fall in LV ejection fraction (mostly in the first 12–24 weeks of therapy), ventricular ectopy, and immune-mediated reactions (83). Thus, to improve enzyme bioavailability in tissues, two strategies have been developed: the use of new rhGAA with high affinity for M6PR and the use of pharmacological adjuvants such as beta2agonists (e.g., clenbuterol and albuterol) or chaperones (e.g., glucose analogs duvoglustat and miglustat) (84).

Given the monogenic origin of Pompe disease, it represents an ideal target for gene therapy. In particular, recent progress has been made in the field of gene therapy mediated by adeno-associated virus vectors (AVV), and several preclinical studies demonstrated the efficacy of this treatment based on the improved clearance of glycogen in the muscle and the enhanced respiratory and cardiac performance. However, the limitations are primarily the high vector doses needed and the transgene immunogenicity caused by the increased muscle-specific GAA expression (84–86).

Danon Disease (Glycogen Storage Disease Type IIb)

Introduction

Danon disease (OMIM code #300257) is a rare X-linked disorder with prominent effects on skeletal and cardiac muscle (87), caused by mutations in the *LAMP2* (88). The real prevalence of this condition is unclear; however, a disease-causing mutation in *LAMP2* has been found in up to 1% of HCM patients (72). LAMP2 is a glycosylated protein that is important in the prevention of the lysosome degradation from its acid hydrolases (89, 90). The impairment of LAMP2 protein function due to gene mutation leads to the accumulation of autophagic material and glycogen (91).

Clinical Presentation and Diagnostic Consideration

Given the X-linked inheritance pattern, the clinical manifestation and the age of presentation are quite different in the two sexes. In males, Danon disease presents at an earlier age and is classically characterized by skeletal myopathy, cardiomyopathy, and intellectual disability.

Danon cardiomyopathy is progressive and usually shows a severe concentric LV hypertrophy (92), which can later progress to LV dilation and heart failure in about 10% of males (93). Conduction abnormalities are also present, manifesting nearly in all of the affected males, and the most common electrocardiographic feature is a short PR interval (preexcitation), present in nearly two thirds of patients (93); the diagnostic flowchart of patients with unexplained left ventricular hypertrophy (LVH) and ventricular preexcitation is shown in **Figure 4**. Skeletal myopathy manifests in 80–90% of males with progressive proximal muscle weakness (93, 94); it is associated with elevated serum creatine kinase (CK) levels, the presence of intracytoplasmic vacuoles containing autophagic material and glycogen, and the absence of LAMP2 protein expression at skeletal muscle biopsy (93). Other common manifestations in males with Danon disease are represented by learning disability and cognitive defects (in 70–100%) and retinal involvement (in 69%) (95).

On the contrary, females with Danon disease are generally less severely affected. Myocardial involvement is present in 60–100% of females, with an equal prevalence of HCM and dilated cardiomyopathy (93); in addition, conduction abnormalities are present in almost all patients (93, 94). Muscle weakness is generally mild to asymptomatic in females, with mild elevation or

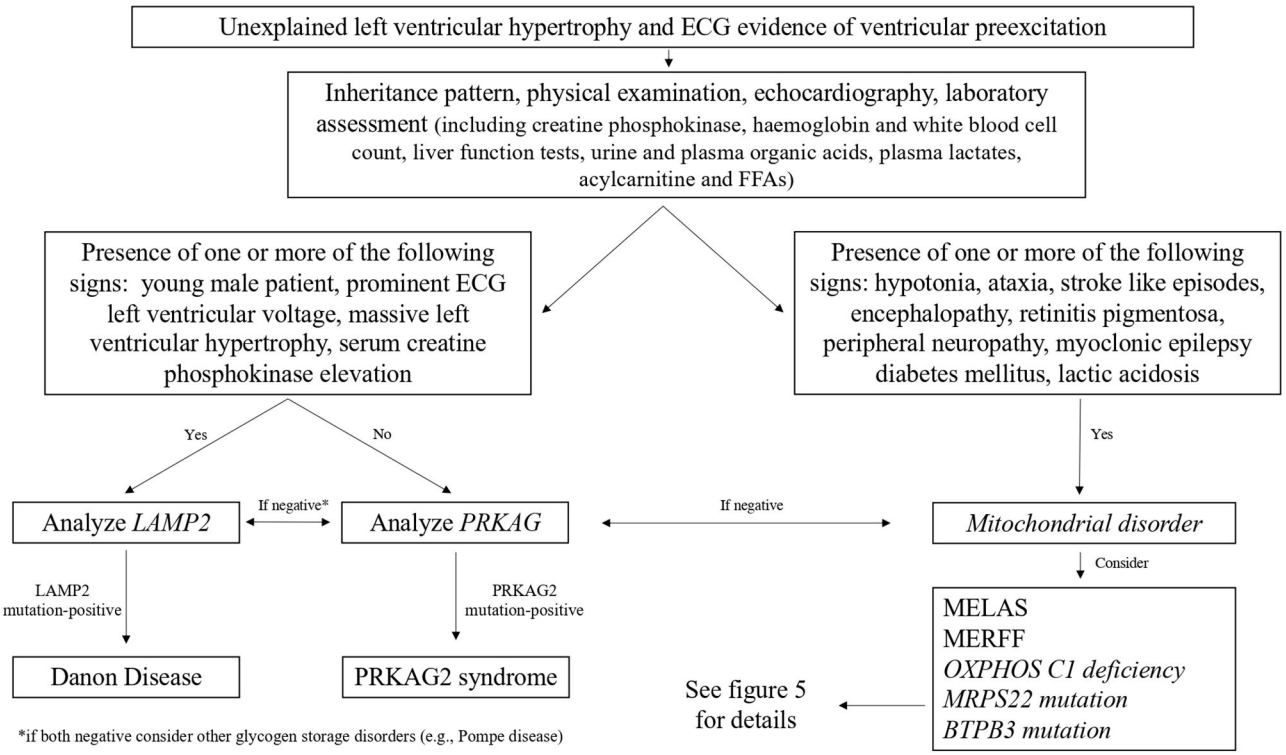

FIGURE 4 | Diagnostic flowchart in patients with unexplained left ventricular hypertrophy and electrocardiographic evidence of ventricular preexcitation. FFA, free fatty acids; MELAS, mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers.

normal serum CK levels (96). Likewise, learning disabilities and cognitive defects are less frequent.

In patients with clinically suspect of Danon disease or unexplained LV hypertrophy, the diagnosis is supported by the presence of normal acid maltase levels on muscle biopsy, the deficiency of LAMP2 protein by immunohistochemistry, the evidence of autophagic vacuole accumulation by microscopy, and the presence of a disease-causing mutation of *LAMP2* (97). Therefore, genetic testing, due to its non-invasive nature and the inclusion of *LAMP2* in HCM genetic testing panels, actually represents the most common tool for identifying Danon disease.

Prognosis and Risk of Sudden Cardiac Death

Heart failure and arrhythmia constitute the leading cause of morbidity and mortality in patients with Danon disease. Recently, data from a multicenter European registry have shown that Danon disease runs a malignant course in both genders, due to cardiac complications (98). In particular, among the 57 patients evaluated (30 males and 27 females), 53% had a heart failure hospitalization, 27% underwent heart transplantation or received an LV assist device, and 30% of patients died during follow-up. Moreover, in this cohort, SCD occurred in three female patients, and appropriate implantable cardioverter-defibrillator (ICD) interventions for ventricular tachycardia/fibrillation were evidenced in six patients, underlying the high risk of SCD in this population (98).

Similar to sarcomeric HCM, SCD in Danon disease is associated with the presence of specific risk factors, including symptomatic arrhythmias, severe hypertrophy, reduced ejection fraction, extended fibrosis on cardiac magnetic resonance (CMR), and family history of SCD (99). However, the association between these risk factors and SCD is based on few studies and with small sample size cohort; thus, the indication for ICD implantation must be individualized on a case-by-case basis.

Treatment

There is no etiological treatment for Danon disease (**Table 3**), and the management should be based on a multidisciplinary team-based approach (95). The management of HCM should follow the current guidelines (1, 9), considering that in patients with Danon disease it may present earlier and may progress more rapidly compared with sarcomeric forms of HCM, in particular in males. Therefore, management with inotropic negative or chronotropic negative agents should be used with caution in Danon disease, considering the possible development of systolic LV dysfunction and/or atrioventricular block (92), which represents possible complications of the disorder.

Recently, Manso et al. (100) tested the efficacy of AAV-9-mediated gene therapy delivering the human LAMP2B gene in a mouse model of Danon disease and showed that gene therapy restored protein expression in multiple organs,

improved metabolic abnormalities and cardiac function, and increased survival.

Cori–Forbes Disease (Glycogen Storage Disease Type III)

Introduction

Among glycogen storage diseases, GSD type III (OMIM code #232400), also called Cori–Forbes disease, is one of the rare disorders of glycogenolysis associated with the development of LV hypertrophy (101). The incidence of GSD type III is ∼1:100,000 in the United States (101). GSD type III is an autosomal recessive disorder caused by *AGL* mutations (102), causing non-functional glycogen debranching leading to the storage of limited dextrin.

Clinical Presentation and Diagnostic Consideration

Two principal forms have been described: GSD type IIIa, characterized by involvement of the liver and the cardiac and skeletal muscle, and GSD type IIIb, by only liver involvement (102, 103). In infants and in children, the principal features are represented by hepatomegaly, hypoglycemia, failure to thrive, recurrent illness, and/or infection (104). Cardiac involvement is common in GSD type IIIa, mostly starting in the first decade of life (104). The International Study on Glycogen Storage Disease (ISGSDIII) (104) showed that cardiac involvement was present in 58% of patients and that nearly two thirds of these patients had electrocardiographic and/or echocardiography signs of LV hypertrophy, whereas the other one third showed a different form of cardiac hypertrophy (i.e., septal, right ventricular, or biventricular hypertrophy). The presence of severe cardiomyopathy is reported in 15% of patients and represents almost the important cause of death in these patients (104, 105). The diagnosis requires liver and/or muscle biopsy or can be formulated through the identification of a disease-causing homozygotic mutation in *AGL* (106).

Treatment

Unfortunately, a specific treatment for GSD III is not currently available, and current treatments are based on symptomatic and dietary management to control blood glucose levels (104, 106, 107). However, due to its monogenic nature, it is a suitable candidate for AAV-mediated gene therapy. Recently, Lim et al. (108) showed that in mouse models of GSD III, the AAV-mediated gene therapy blocked the glycogen accumulation in both cardiac and skeletal muscle and improved the muscle functions.

PRKAG2 Syndrome

Introduction

PRKAG2 syndrome is a metabolic disorder, inherited with an autosomal dominant pattern, which is characterized by LV hypertrophy, conduction abnormalities, and ventricular preexcitation, caused by mutations in *PRKAG2* (71, 109). *PRKAG2* mutation is reported in nearly 1% of patients with HCM (72) and in 29% of those with both LV hypertrophy and preexcitation on ECG (110, 111). *PRKAG2* encodes for the γ2 regulatory subunit of AMP-activated protein kinase (AMPK) (112). *PRKAG2* mutation leads to structural changes of AMPK (113–115), leading to impaired myocyte glucidic uptake, which results in intracellular glycogen and amylopectin deposition, finally causing storage cardiomyopathy (116). PRKAG2 mutations have also been linked to conduction abnormalities and ventricular preexcitation; however, the underlying mechanism of this association is still unclear (117).

Clinical Presentation and Diagnostic Consideration

PRKAG2 syndrome may present different severity degree of both the ventricular hypertrophy and arrhythmic manifestations (118). The onset of symptoms commonly occurs within the first three decades of life and arrhythmic symptoms (i.e., palpitations, lipotomies, and syncopal episodes) represent the most common disease presentation (72).

The most common electrocardiographic feature of PRKAG2 syndrome is a short PR interval, found in nearly two thirds of patients (72) (**Figure 4**); other electrocardiographic abnormalities are represented by the right bundle branch block, different patterns of intraventricular conduction abnormalities, and sinoatrial blocks (119). LVH, evidenced thought echocardiography or CMR, often showed an eccentric distribution with a progressive wall thickness increase in the large part of affected individuals (72). CMR is important in early detecting myocardial involvement. In the early phase of the disease, a focal mid-infero-lateral pattern may be present, and TI values may be reduced, while a diffuse myocardial involvement is generally evidenced in patients with an advanced phase of the disease, and the presence of fibrosis causes T1 value elevation (109).

In the advanced phase, evolution forward LV dilation and dysfunction are possible, with the subsequent development of heart failure (120). Supraventricular tachyarrhythmias were evidenced in more than one third of PRKAG2 syndrome patients, and the large part of these showed an accessory pathway on the electrophysiological study (119, 121, 122). They are mainly represented by atrial fibrillation and flutter; and their complications are represented by stroke and ventricular arrhythmias, sometimes leading to SCD (72, 119, 122). Conduction system dysfunctions, such as advanced atrioventricular blocks, marked sinus bradycardia, and sinus blocks are present in about half of PKAG2 syndrome patients, leading, in a large part of them, to pacemaker implantation (1, 112). The family screening and, when indicated, genetic testing are of great importance for diagnosis.

Prognosis and Risk of Sudden Cardiac Death

Heart failure development and arrhythmia, and both bradyarrhythmia and tachyarrhythmia, constitute the leading cause of morbidity and mortality in patients with PRKAG2 syndrome. SCD occurs in about 10% of PRKAG2 syndrome patients, mainly as a consequence of advanced atrioventricular block or supraventricular tachycardia degenerated in ventricular

fibrillation, or from massive hypertrophy (72, 123, 124). However, current data are not sufficient to clearly define the precise pathophysiologic process leading to SCD.

Treatment

Given the numerous life-threatening consequences of PRKAG2 syndrome, early identification and management of its complications is mandatory. Actually, no specific guidelines have been formulated for PRKAG2 syndrome. The management of HCM should follow the current guidelines (1, 9). Pacemaker implantation is recommended in patients with symptomatic sinus node dysfunction or atrioventricular block. ICD implantation in primary prevention should be performed after a careful evaluation of the individual risk factors, such as familial history of SCD, arrhythmic syncopal episodes, severe LV hypertrophy, non-sustained ventricular tachycardia, and extended fibrosis on CMR (4, 119).

LYSOSOMAL STORAGE DISEASES

Lysosomal storage diseases are a heterogeneous group of inherited disorders characterized by the accumulation of undigested or partially digested macromolecules, leading to cellular dysfunction and organomegaly. The forms that most commonly cause HCM are represented by mucopolysaccharidoses (MPSs).

Mucopolysaccharidoses

Introduction

The MPSs (OMIM code #252700) are a heterogeneous group of lysosomal storage disorders caused by the functional deficiency of one of the lysosomal enzymes involved in the catabolism of glycosaminoglycans (GAGs) (125). Individuals with MPSs are affected by progressive deposition of incompletely degraded GAGs within potentially in all organ systems, although the specific distribution depends on the specific disease. Except for MPS type II, which is inherited with an X-linked recessive pattern, all the MPSs are inherited with autosomal recessive pattern (126).

Clinical Presentation and Diagnostic Consideration

Typical manifestations include skeletal and joint deformities, growth and intellectual disability, central nervous system involvement, respiratory difficulty, gastrointestinal disorders, cardiovascular diseases, and ocular and hearing alterations (127). Cardiovascular involvement is generally present, and it occurs earlier and more frequently in MPS type I, type II, and type VI. Cardiac involvement in MPSs is caused by the massive accumulation of dermatan sulfate especially into valves and great vessels, where this GAG is normally present in a large amount. Its deposition leads to the infiltration of granular cells into valves, myocardial walls, coronary arteries, and conduction system inducing inflammation mediated by Toll-like 4 receptor pathway (128). The principal expression of cardiac involvement is represented by valvular regurgitations and stenosis, mainly involving the left-sided valves, caused by thickening of leaflets or papillary muscles and shortening of subvalvular apparatus. The presence of HCM, endocardial thickening, dilated cardiomyopathy with reduced ejection fraction, and pulmonary hypertension has also been described (129, 130). Typically, LV hypertrophy and diastolic dysfunction occur in the early phases, while LV dilation and systolic dysfunction are common in the final disease stage. Moreover, coronary artery disease and systemic hypertension are common in patients with MPSs and have been associated with the diffuse intimal proliferation from GAG deposition, while electrophysiological anomalies such as atrioventricular blocks are related to fibrosis of the conduction system (128, 131, 132).

The enzyme activity assays on fibroblasts, leucocytes, or serum are the gold standard for a definitive diagnosis. Gene analysis can identify the mutations present; in particular, homozygous mutation in the gene encoding alpha-L-iduronidase (*IDUA*) is diagnostic for MPS type I, in the gene encoding iduronate-2-sulfatase (*IDS*) for MPS type II, and in the gene encoding arysulfatase B (*ARSB*) for MPS type VI (125).

Prognosis and Risk of Sudden Cardiac Death

Regardless of phenotype, all forms of MPS are associated with increased morbidity and mortality. However, the prognosis of patients with MPS mainly varies according to the type of MPS and the residual level of the deficient enzyme. For example, in MPS I H, the most severe phenotype, patients often die in the first decade of life for infectious, respiratory, or cardiac complications.

Cardiovascular cause of death is mainly caused by advanced heart failure, while SCD or death from coronary occlusion is rare (133, 134).

Treatment

In MPS patients, cardiac surgery for valve disease is often performed with success, and standard pharmacological therapy for heart failure management is commonly used in current clinical practice (128). Actually, ERT and hematopoietic stem cell transplantation (HSCT) represent the standard of care for most MPS diseases (**Table 3**). ERT, approved for MPSs I, II, IV, VI, and VII, has improved pulmonary function, walking ability, muscular pain, and organomegaly. This treatment has shown a significant increase in systolic and diastolic functions and an important hypertrophy regression. However, ERT and HSCT are ineffective on poorly vascularized sites such as cardiac valves, corneas, and cartilage (128). The most common adverse events with ERT are IgG antidrug antibody formation and infusion-related hypersensitivity reactions (135–137). HSCT is used in all forms of MPS except MPS III, even if several studies show important benefits of this treatment only in MPS I. Its major adverse effects are graft vs. host disease (GVHD) and graft rejection. Beneficial effects are the significant increase of long-term survival, interruption of pulmonary and cardiac disease progression, especially regarding coronary artery occlusion and ventricular hypertrophy, and the attenuation of some neurocognitive symptoms thanks to its ability to cross the brain–blood barrier (BBB) (138). In contrast to ERT, HSCT is considered a permanent treatment. Recently, the association

between ERT and HSCT seems to decrease the likelihood of GVHD and improve therapeutic efficacy (139–141). Among new approaches actually undergoing testing, there is gene therapy. This treatment, based on the direct infusion of viral vectors expressing the functional gene (*in vivo* gene therapy) or on the infusion of modified and transduced cells form recipient patient (*ex vivo* therapy), has the advantage to promote continuous enzyme secretion and to be a permanent therapy (142). Pharmacological chaperone therapy aims to provide the correct folding and the highest stability possible of the mutant proteins, avoiding their deposition and aggregation. These little molecules cross BBB and are not immunogenic, but they can present off-target adverse effects and are not currently approved for MPS disorders (143).

MITOCHONDRIAL DISORDERS

Introduction

Mitochondrial disorders represent an extremely heterogeneous group of disorders caused by the dysfunction of the mitochondrial respiratory chain. Several proteins are responsible for the integrity of the mitochondrial structure and function, with the major part encoded by the nuclear DNA (nDNA) and the minor part by the mitochondrial DNA (mtDNA) (144).

For this reason, mitochondrial disorders are a challenging area of genetics (144, 145); indeed, this condition results in different possible inheritance patterns of mitochondrial disorder (i.e., autosomal dominant, autosomal recessive, X-linked, and maternal). Mutations in mtDNA or nDNA genes result in mitochondrial dysfunction, thus resulting in mitochondrial disorders (146). Mitochondrial disorders are typically multiorgan disorders, especially involving those organs with high-energy requirement (147), and it can clinically manifest in the neonatal phase, childhood, or adulthood (148).

Because cardiac muscle is one of the high-energy-demanding tissues, the myocardial involvement (i.e., mitochondrial cardiomyopathy) occurs in about 20–40% of children with mitochondrial disease (147), and it can present as an isolated feature or part of a multiorgan involvement (149). HCM is the most common form of mitochondrial cardiomyopathy; however, other forms of cardiomyopathies are possible.

Clinical Presentation and Diagnostic Consideration

Several mitochondrial syndromes showed a myocardial involvement as a part of their multiorgan spectrum (149). In these conditions, typically associated with mtDNA mutations, myocardial involvement is characterized by cardiomyopathy and/or conduction abnormalities. In particular, myocardial involvement may be associated with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS syndrome) (150); myoclonic epilepsy with ragged red fibers (MERRF syndrome) (151); and neurogenic muscle weakness with sensory neuropathy, ataxia, and pigmentary retinopathy (NARP syndrome) (152). Clinical presentation is extremely variable, with the early onset generally associated with worse outcome and the later onset with mild clinical presentation (63).

Next to the classical mitochondrial syndrome, several other mitochondrial disorders associated with HCM have been identified and can be classified in mitochondrial disorders caused by single or multiple respiratory chain complex deficiencies (153), CoQ10 deficiency (154), mitochondrial transporters deficiency, disorders characterized by 3-methylglutaconic aciduria (e.g., Barth syndrome), disorders of mitochondrial iron metabolism (e.g., Friedreich ataxia), and disorders of mitochondrial β-oxidation and carnitine metabolism (149) (**Figure 5**).

Deficiency of individual or multiple respiratory chain might result from a mutation in mtDNA or nDNA mitochondrial-related genes, and in both these conditions, it is possible that cardiomyopathy manifests as an isolated feature or in the spectrum of a multisystem disorder (153).

A comprehensive diagnostic workup is required to obtain a final diagnosis (**Figure 5**). The inheritance pattern, the pattern of organ involvement, the presence of specific findings on clinical and instrumental evaluation, and typical biochemical abnormalities might narrow the differential diagnosis.

Specific Forms: Barth Syndrome

Barth syndrome (OMIM code #302060) is a rare X-linked genetic disease due to mutation in TAZ gene, which encodes for tafazzin, a phospholipid transacylase that plays an important role in the remodeling of cardiolipin. Cardiomyopathy is present in nearly 70% of Barth syndrome patients and appears in the 1st year of age, usually manifesting as dilated cardiomyopathy or LV non-compaction; HCM appears to be rarer (155, 156). Other clinical manifestations include skeletal myopathy, growth delay, neutropenia, and increased urinary excretion of 3-methylglutaconic acid (3-MGCA) (157).

Specific Forms: Friedreich Ataxia

Friedreich ataxia (OMIM code #22930) is a neurodegenerative disorder, inherited with an autosomal recessive pattern, caused by the homozygous GAA triplet repeat expansion in the *FXN*, which encodes for the protein frataxin. The first symptoms usually appear in the second decades of life, and the clinical presentation includes progressive ataxia, dysarthria, peripheral neuropathy, and diabetes mellitus (158). Cardiovascular involvement, usually manifesting as unexplained LVH appear during adolescence and is present in nearly two thirds of patients; however, in some cases, the disease can progress to progressive LV dilatation and dysfunction, which can result in heart failure and cardiovascular death.

Treatment

To date, the only mitochondrial disorders with an etiologic treatment are those caused by CoQ10 deficiency and thiamine-responsive disorders (159) (**Table 3**). However, target therapies are present for specific mitochondrial diseases. For monogenic disorder, one attractive strategy is AAV gene replacement therapy. For example, in mouse models of Barth syndrome, the AAV-mediated gene therapy rescued neonatal demise, prevented cardiac dysfunction, and reversed established heart disease (160),

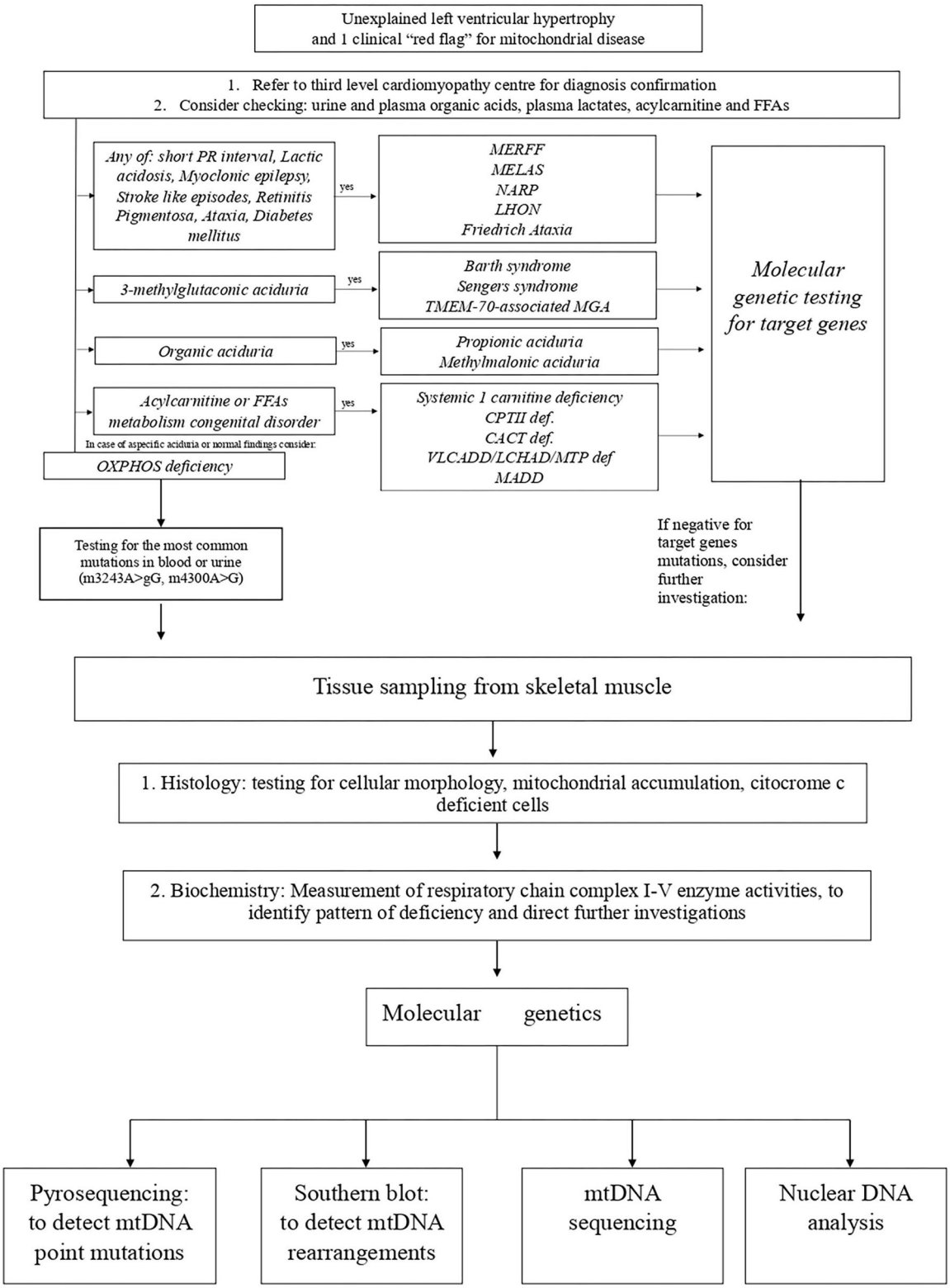

FIGURE 5 | Proposed algorithm for the diagnosis of mitochondrial cardiomyopathies. CACT, carnitine-acylcarnitine translocase; CTPII, carnitine palmitoyltransferase II; FFA, free fatty acids; LCHAD/MTP, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; LHON, Leber's hereditary optic neuropathy; MADD, multiple-acyl-CoA dehydrogenase deficiencies; MELAS, mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers; MGA, methylglutaconic aciduria; NARP, neurogenic muscle weakness with sensory neuropathy; VLCADD, very-long-chain acyl-CoA dehydrogenase deficiency.

suggesting that gene therapy might be a therapeutic option for patients with Barth syndrome.

In patients with mitochondrial disorders, heart transplantation may be considered in patients with severe isolated cardiomyopathy or when the eventually extracardiac manifestations are mild and appear non-progressive (161).

CONCLUSIONS

HCM in children represents a large heterogeneous group of disorders. A comprehensive approach, including medical history, physical examination, detailed cardiac imaging, and attention to possible extra-cardiac abnormalities, is required to suspect a specific disorder and to perform a specific diagnosis. The early referral to third-level cardiomyopathy center may be useful in order to start tailor management and potentially initiate targeted genotype-based therapies for these rare conditions.

AUTHOR CONTRIBUTIONS

EM, MR, and GL contributed to the conception and design of the work. EM, MR, ML, FDF, RP, FV, and GL drafted the manuscript. All the authors critically revised the manuscript. All the authors gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy. All the authors have read and agreed to the published version of the manuscript.

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CASE REPORT
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Case Reports: Emery-Dreifuss Muscular Dystrophy Presenting as a Heart Rhythm Disorders in Children

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Emery-Dreifuss muscular dystrophy (EDMD) is inherited muscle dystrophy often accompanied by cardiac abnormalities in the form of supraventricular arrhythmias, conduction defects and sinus node dysfunction. Cardiac phenotype typically arises years after skeletal muscle presentation, though, could be severe and life-threatening. The defined clinical manifestation with joint contractures, progressive muscle weakness and atrophy, as well as cardiac symptoms are observed by the third decade of life. Still, clinical course and sequence of muscle and cardiac signs may be variable and depends on the genotype. Cardiac abnormalities in patients with EDMD in pediatric age are not commonly seen. Here we describe five patients with different forms of EDMD (X-linked and autosomal-dominant) caused by the mutations in *EMD* and *LMNA* genes, presented with early onset of cardiac abnormalities and no prominent skeletal muscle phenotype. The predominant forms of cardiac pathology were atrial arrhythmias and conduction disturbances that progress over time. The presented cases discussed in the light of therapeutic strategy, including radiofrequency ablation and antiarrhythmic devices implantation, and the importance of thorough neurological and genetic screening in pediatric patients presenting with complex heart rhythm disorders.

Keywords: Emery-Dreifuss muscular dystrophy, children, atrial tachycardia, atrial fibrillation, cardiomyopathy, *LMNA*, *EMD* (emerin), pacemaker implantation

INTRODUCTION

Emery-Dreifuss muscular dystrophy (EDMD) is a group of inherited muscle-joint-cardio syndromes (1). The cardiac involvement in EDMD can be severe and life-threatening (2). Various genetic backgrounds contribute in different ways to the broad spectrum of cardiac manifestations, mainly in the form of conduction disorders, supraventricular arrhythmias and more rarely functional and structural abnormalities of the heart (3).

EDMD is a rare disease. The general prevalence of EDMD is 0.39–1 per 100,000, with significant heterogeneity of estimates; in the pediatric population −0.22 per 100,000 (4–6). The classic clinical triad includes early joint contractures (elbows, neck, ankles, and spine), slowly progressive muscle weakness/atrophy and cardiac abnormalities, but the clinical course and sequence of symptoms depend on the genotype. The severity of cardiac involvement does not correspond to the progression of muscular weakness (3). In the case of EDMD1, associated with mutations in the *EMD* gene, the contractures frequently emerge in the first decade of life, become more significant during the growth spurt followed by muscle atrophy and weakness in the second decade of life and usually precede cardiac phenotype. In EDMD2, caused by mutations in the *LMNA* gene, symptoms varied widely from a mild phenotype with a later onset and slow progression to a severe one with life-threatening complications. Lack of information for the reported cases EDMD3, EDMD4, and EDMD5 linked to the genes encoding proteins of nuclear envelope does not allow to define a unified clinical picture of each of those subtypes (7, 8).

Cardiac involvement in EDMD usually follows muscle phenotypes and predominantly presents after the second decade of life (9, 10). After the diagnosis is established due to typical muscular manifestation, the cardiac abnormalities are screened in the frame of expected disease phenotype (11). Several cases of early cardiac debut preceding muscle dystrophy have been reported, and even isolated cardiac involvement has been described; still, these cases remain scarce and atypical (10, 12–15). The cardiac disease commonly manifests from atrial arrhythmias and conduction disturbances. Syncope and sudden cardiac death (SCD), caused by complete heart block or ventricular tachyarrhythmia, can also occur, and single cases of left ventricular non-compaction have been described (16, 17). Systolic dysfunction and dilated cardiomyopathy found in a minority of patients and are mainly associated with autosomal-dominant disease (AD-EDMD) due to *LMNA*-mutations (18–22). Creatine kinase (CK) level can range from normal to 15 times the upper limit, without direct correlation with muscular and cardiac involvement, so in patients with severe cardiac phenotype CK levels could remain normal (23).

The genetic spectrum of EDMD includes mutations in *EMD*, *LMNA*, *SYNE1*, *SYNE2*, *FHL1*, *TMEM43*, *SUN1, SUN2,* and *TTN* genes (5, 24–28). These genes mainly encode for the nuclear envelope proteins, which give rise to the term "nuclear envelopathies" (5, 23, 29). An exception is FHL1 protein which localizes to the sarcomere and the sarcolemma but may also shuttle between cytosolic and nucleoplasmic fraction, thus becoming a part of the nuclear envelope (30). Mutations in *LMNA* and *EMD* are the most common causes of EDMD, and together they account for about 36% of the cases (5). Thus, for EDMD, there are still a number of undetected causative genes (30). An AD-EDMD mainly arises from mutations in *LMNA*, which contribute to ∼28% of the cases. In *LMNA*-associated EDMD, males and females are equally affected and left ventricular dysfunction is more commonly observed as well as ventricular arrhythmias and SCD (9, 19, 20). Several cases of *LMNA*-associated autosomal-recessive form of EDMD presented in pediatric patients have also been described (31, 32). The X-linked form is usually caused by mutations in the *EMD* gene (8%) and, rarely, in the *FHL1* gene (2%) and predominantly affects males with rare cases of disease manifestation in female carriers (18). Mutations in the *EMD* gene occur sporadically and rarely, but mutations in the *LMNA* gene are increasingly identified (23).

Due to the typical presentation during the second-third decades of life and a debut from muscle symptoms, there are only several reports on EDMD presented in children with the isolated cardiac phenotype (13, 14, 33–35). Here we describe five cases of EDMD1 and EDMD2 with a cardiac manifestation in childhood and discuss the need for target screening of neuromuscular phenotypes in children with unexplained atrial dysfunction, conduction abnormalities, and ventricular arrhythmias.

MATERIALS AND METHODS

Patients were examined between 2009 and 2020 in tertiary pediatric cardiac care center – Almazov National Medical Research Centre, St. Petersburg, Russia. All data, including the clinical history, case notes, reports of instrumental methods and surgical protocols were extracted from paper and electronic databases. Clinical examination included physical, 12-lead electrocardiography (ECG), Holter monitoring (HM), transthoracic echocardiography, neurological examination and laboratory tests. Additionally, we performed CMR, electrophysiological study (EPS) and electroneuromyography (ENMG). One patient underwent an endomyocardial biopsy to exclude inflammatory heart disease.

GENETIC ANALYSIS

Target sequencing was performed using a panel of 108 or 172 genes, as previously described (36). For Patients 1 and 2, a targeted panel of 108 cardiomyopathy-associated genes has been initially studied using Haloplex Target Enrichment System (Agilent, Waldbronn, Germany) with an Illumina MiSeq instrument (for gene list, see **Supplementary Table 1**). For Patients 3–5, a targeted panel of 172 cardiomyopathy-associated genes was studied using the SureSelect Target Enrichment System (Agilent; Waldbronn, Germany) (see **Supplementary Table 2**). Data processing and variant calling were performed according to GATK BestPractice recommendations (Broad Institute, Cambridge, MA, USA) using hg19 and hg38 human genome references. For variant validation, bidirectional Sanger sequencing was performed using ABI 3500 machine (Applied Biosystems). All novel and previously reported variants of interest with a frequency below 0.01% were classified according to the recommendations of American College of Medical Genetics (37). The described variants were submitted to Gene bank repository under submission numbers SCV001548550 - SCV001548554.

TABLE 1 | Clinical and genetic characteristics of patients affected by EDMD and presented with heart rhythm disorders.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Mutation variant	**EMD** NM_000117.3:c.631delC (pArg211ValfsTer26)	**EMD** NM_000177.3: c.449+1G>A	**EMD** NM_000117.3: c.173C>T (p.Ser58Phe) rs781797234	**LMNA** NM_170707.4: c.746G>A (p.Arg249Gln) rs59332535	**LMNA** NM_170707.3: c.305T>C (p.Leu102Pro) rs1553262
Age at first cardiac manifestation	14 y.o.	11 y.o. (possibly earlier)	16 y.o.	16 y.o.	9 y.o.
Heart rhythm disorders	AT, AF, AFib, PACs, AVNRT, SSNS, AVB II	AT, PACs, SSNS	SB, AVB I, II	AFib, AF, PACs, PVCs, SAB II, AVB I, II	AT, AF, AFib, PACs, SSNS, AVB I, II
Inheritance	Maternal grandfather – SCD, DCM (31 y.o) Maternal cardiac examination showed no pathology	No data	Brother had aborted cardiac arrest at 1 y.o. Maternal cardiac examination showed no pathology	No Similar mother's DNA pathology wasn't detected	Parents have no ECG pathology.
Current age	20 y.o.*	18 y.o.*	28 y.o.	22 y.o.	15 y.o.
Structural and functional cardiac abnormalities	LA dilatation (z-score** 3.04) and LV EF 54%	RA dilation (z-score 2.72),* RV dilation (z-score 2.98)	No	No	No
Antiarrhythmic therapy	Metoprolol tartrate, Lappaconitine hydrobromide, Sotalol, Propafenone	Lappaconitine hydrobromide	No	Metoprolol tartrate	Metoprolol tartrat, Propafenone
Other therapy	Perindopril	Perindopril Spironolactone	No	No	No
Clinical symptoms	Palpitations	Palpitations	Dizziness	Palpitations, reduced physical tolerance	Palpitations, reduced physical tolerance
Syncope	No	No	No	No	No
Neuromuscular phenotype	Not in childhood Mild myopathic changes in the upper limbs by ENMG after 18 y.o.	No	Not in childhood elbow contractures presented at 25 y.o	Myopathic changes since 1.5 y.o. Detected by ENMG	elbow and ankle contractures, muscle weakness in the upper limbs since 11 y.o.
Pacemaker implantation	16 y.o.	Patient's refusal	No	No	15 y.o.
RFA	AVNRT (eff) AF (eff)	Multifocus AT (no eff)	No	No	No
CK level	Normal	Normal	↑CKx2	↑CKx7	↑CKx6,5

AT, atrial tachycardia; AF, atrial flutter; AFib, atrial fibrillation; AVB, atrioventricular block; AVNRT, atrioventricular nodal reentrant tachycardia; CK, creatine kinase; EF, ejection fraction; eff, efficiency; ENMG, electroneuromyography; LA, left atrium; LV, left ventricle; PACs, premature atrial captures; PVCs, premature ventricular contractions; RFA, radiofrequency ablation; SSNS, sick sinus node syndrome; RV, right ventricle; SAB, sinoatrial block; SB, sinus bradycardia.

**z-score values were used for assessment of dilatation by echocardiography.*

***LV dysfunction was considered as a reduction of LV ejection fraction < 55% (Simpson method).*

ETHICAL CONSIDERATIONS

The study was performed according to the Declaration of Helsinki, and approval was obtained from the local ethical committee of Almazov National Medical Research Centre. Written informed consents were obtained from the parents of the minor prior to investigation.

RESULTS

We observed five patients with EDMD and cardiac symptoms in childhood: three with EDMD1 and two with EDMD2. All patients were males. The mean age of cardiac manifestation was 13.2 ± 3.11 (from 9 to 16 y.o.). The mean follow-up period was 7.4 ± 2.6 years. All patients had sinus node dysfunction and four out of five - atrioventricular block (AVB). The leading arrhythmic phenotypes included various types of supraventricular arrhythmias: multifocal atrial tachycardia (mAT) ($n = 4$), premature atrial captures (PACs) ($n = 4$), atrial flutter (AF) ($n = 3$), atrial fibrillation (AFib) ($n = 3$) and AV nodal recurrent tachycardia (AVRNT). Arrythmias were the first manifestation in four patients. Patients predominantly complained of palpitation ($n = 4$), dizziness ($n = 1$), fatigue, and reduced physical tolerance ($n = 2$) (**Table 1**).

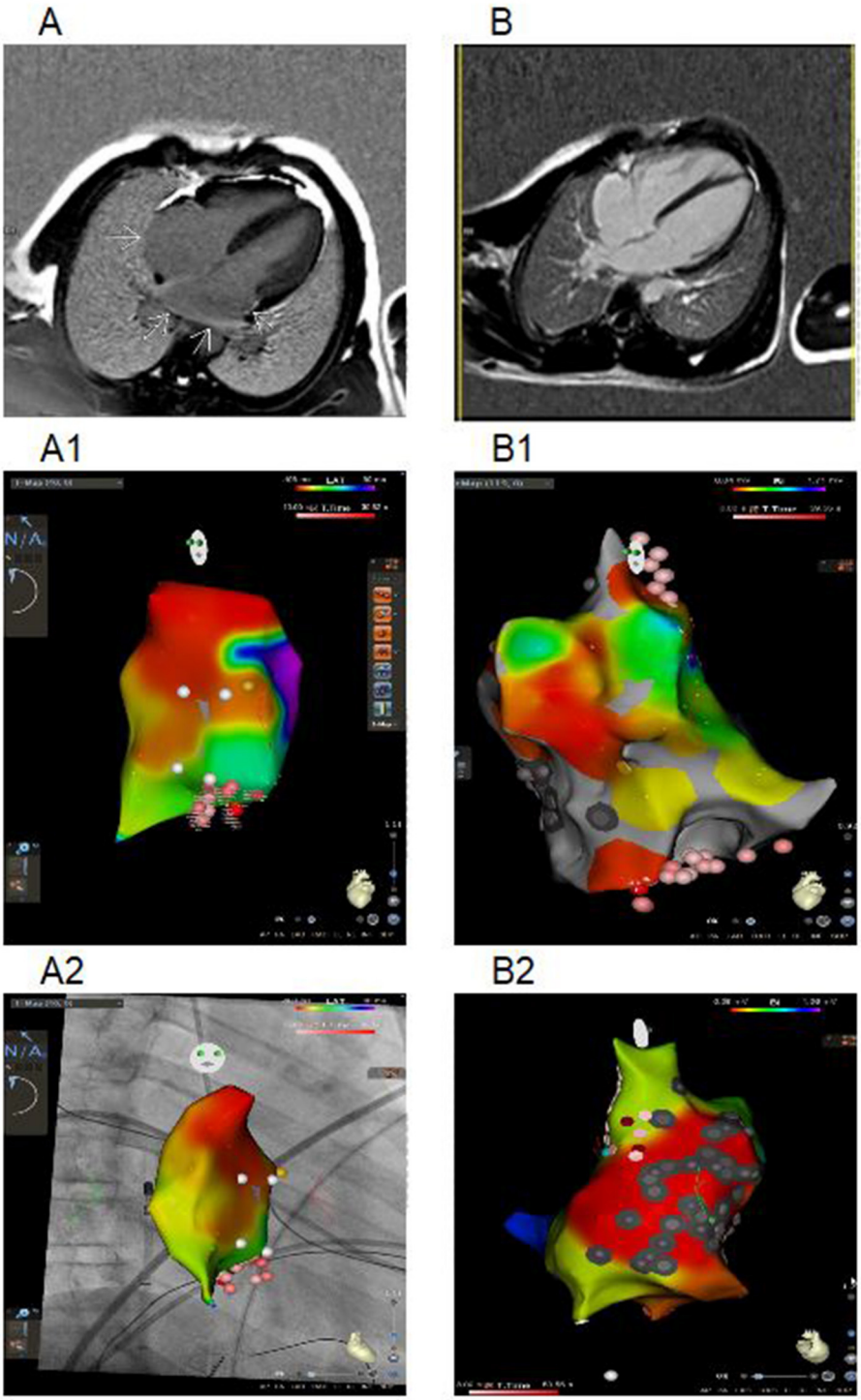

FIGURE 1 | Cardiac maps using three-dimensional mapping system and CMR (imaging in the 4-chamber plane) of Patient 1 and 2. **(A)** CMR from a patient 1: delayed-enhancement CMR shows areas of myocardial scarring in both atria (arrows). **(A1)** Right atrial activation map (CARTO 3 system), LAO position. White dots – location points, yellow dots – part of conduction system, red and pink dots – RF ablation points (the degree of color intensity depends on the RF exposure time). **(A2)** Right atrial activation map (CARTO 3 system with CARTOUNIVU module), RAO position. White dots – location points; yellow dots – part of conduction system, red and pink dots – RF ablation points (the degree of color intensity depends on the RF exposure time). **(B)** CMR from a patient 2 with RA dilatation. **(B1)** Bipolar map of right atrium (CARTO 3 system), LAO position. **(B2)** Bipolar map of right atrium (CARTO 3 system), non-standard position with focus on the scar and ablation sites. Gray dots – scar points, white dots – location points, blue dots – double potential, red and pink dots – RF ablation points (the degree of color intensity depends on the RF exposure time). Bipolar color scale: red fill color has the lowest voltage.

Patient 1

The patient first presented with atrial rhythm and rare PACs on the ECG at the age of 14 years. He had no complaints, was a member of a football team and was undergoing ECG annually. No pathology was noticed on echocardiography. At 14.5 y.o. he complained on palpitations. An EPS revealed three types of arrhythmias: non-sustained mAT with heart rate (HR) 114 bpm, non-sustained AF and slow-slow AVRNT with HR 130 bpm. The radiofrequency ablation (RFA) of AVRNT was performed. Metoprolol tartrate was prescribed but canceled due to progressive AVB with pauses up to 3,261 ms. Six months later, AF with irregular AV conduction (2:1–4:1) was registered and followed by to RFA of the inferior vena cava-tricuspid isthmus. During the procedure, extensive low-amplitude areas corresponding to fibrosis fields were observed in the right atrium (**Figure 1**). CMR showed mild-dilated right atrium, atrial fibrosis and no acute inflammatory (**Figure 1**). The progression of the sinus and AV nodes dysfunction with bradycardia to 34–43 bpm and pauses to 7,104 ms (**Figure 2**), increasing atrial ectopy led to dual-chamber pacemaker (PM) implantation and Propafenone therapy. Increasing of pacing percentage, no intrinsic rhythm, non-sustained AFib during PM programming registered over time. Target genetic screening using 108-gene panel identified novel genetic variant in *EMD* gene (NM_000117.3):c.631delC, p.Arg211ValfsTer26 (chrX:153609422) classified as pathogenic according to ACMG criteria (PVS1, PM2, PP3). Parental genetic testing hasn't been performed. CK level was not elevated. Neurological examination showed no pathology, but mild myogenic changes in the upper limbs were detected by ENMG later, at the age of 18 years. Maternal grandfather died suddenly at the age of 31 with dilated cardiomyopathy according to the autopsy. Maternal cardiac examination revealed no pathology.

Patient 2

Patient 2 was diagnosed with atrial rhythm with 93 bpm during ECG before being brought to the orphanage at the age of 11 years. His family history is unknown. He has been abusing nicotine and alcohol since the age of 15. On ECG, at the age of 14, atrial and junctional rhythm with PACs and non-sustained mAT were registered. Six months later, he was hospitalized in the emergency unit with palpitations. AT with HR to 200 bpm were registered and aborted by overdriving stimulation. Echocardiography and CMR confirmed right chambers dilatation without myocardial dysfunction. No signs of acute myocardial damage or fibrosis have been found (**Figure 1**). HM identified sinus bradycardia to 35 bpm, mainly atrial and junctional rhythm, mAT with variable AV conduction (2:1–7:1) and pauses to 4,404 ms (**Figure 2**). The RFA of mAT was performed (**Figure 1**) but wasn't effective. Endomyocardial biopsy showed no myocarditis signs. Target genetic screening using 172-gene panel identified *EMD* genetic variant (NM_000117.3):c.449+1G>A genetic variant, classified as pathogenic according to ACMG criteria (PVS1, PM2, PP3) previously reported in association with EDMD (38). He had not myopathy signs or contractures. ENMG was not performed due to the patient's unwillingness. CK level was normal. PM implantation was recommended, but the patient refused. Attempts to prescribe antiarrhythmic therapy (AAT) were unsuccessful.

Patient 3

Patient 3 first consulted a cardiologist at the age of 16 because of dizziness and fatigues. No structural abnormalities were detected by echocardiography. Rare symptomatic episodes of resting bradycardia to 30–34 bpm and pauses due to AVB II up to 2,500 ms were observed. Neurological examination didn't reveal any pathology. Later, at the age of 25, mild elbow contractures were noted. Two times higher CK level was documented at the age of 28. Genetic screening identified a variant in *EMD* (NM_000117.3):c.173C>T (p.Ser58Phe), classified as a variant of unknown significance according to ACMG criteria. Brother survived the aborted cardiac arrest at the age of 1 year, but his genotyping is currently infeasible. Maternal cardiac examination showed no pathology.

Patient 4

The patient was first referred to a neurologist at 1.5 years of age due to toe-walking. He had two times elevated CK level and myopathic changes detected by ENMG. Multifocal PACs were registered at the age of 16 years when the fatigue and palpitations appeared. A HM showed paroxysms of non-sustained AFib, multiple PACs, and transient AVB II and SA block. No pathology was detected on echocardiography and CMR. The CK level has been increased seven-fold. He had ankles contractures, muscle weakness in the shoulders and lower legs. Metoprolol tartrate was initiated with good response and without conduction worsening. Three months later, atrial arrhythmias were increased and rare premature ventricular contractions (PVCs) were registered. Metoprolol tartrate dose was doubled with a positive effect. The genetic investigation identified *LMNA* (NM_170707.4):c.746G>A (p.Arg249Gln) variant previously described in EDMD patients (rs59332535) and classified as pathogenic according to ACMG criteria. Mother doesn't have this variant, father died at 49 y.o. during a planned surgery due to cardiac arrest, no DNA was available for the genotyping. This patient has not indications for PM or cardioverter-defibrillator implantation (ICD).

Patient 5

The patient was first examined by a cardiologist at nine y.o. due to palpitations, but ECG revealed no pathology, HM and echocardiography were not performed. At the age of 11 episodes of mAT, PACs, and transient AVB I were detected by HM. Concurrently, elbow contractures and muscle weakness were noted. ENMG identified a moderate myopathic pattern in the lower limbs. Increase in CK by 6.5 times was detected. Within the next several years, arrhythmias progressed with the sustained episodes of mAT, AFib, deterioration of AV conduction and decrease of HR. Specific changes in the P-wave were detected (**Figure 3**). No structural abnormalities were detected by echocardiography. Metoprolol tartrate was up-titrated with a good but temporary effect. The genetic study identified an earlier reported variant in the *LMNA* gene (NM_170707.3):c.305T>C,

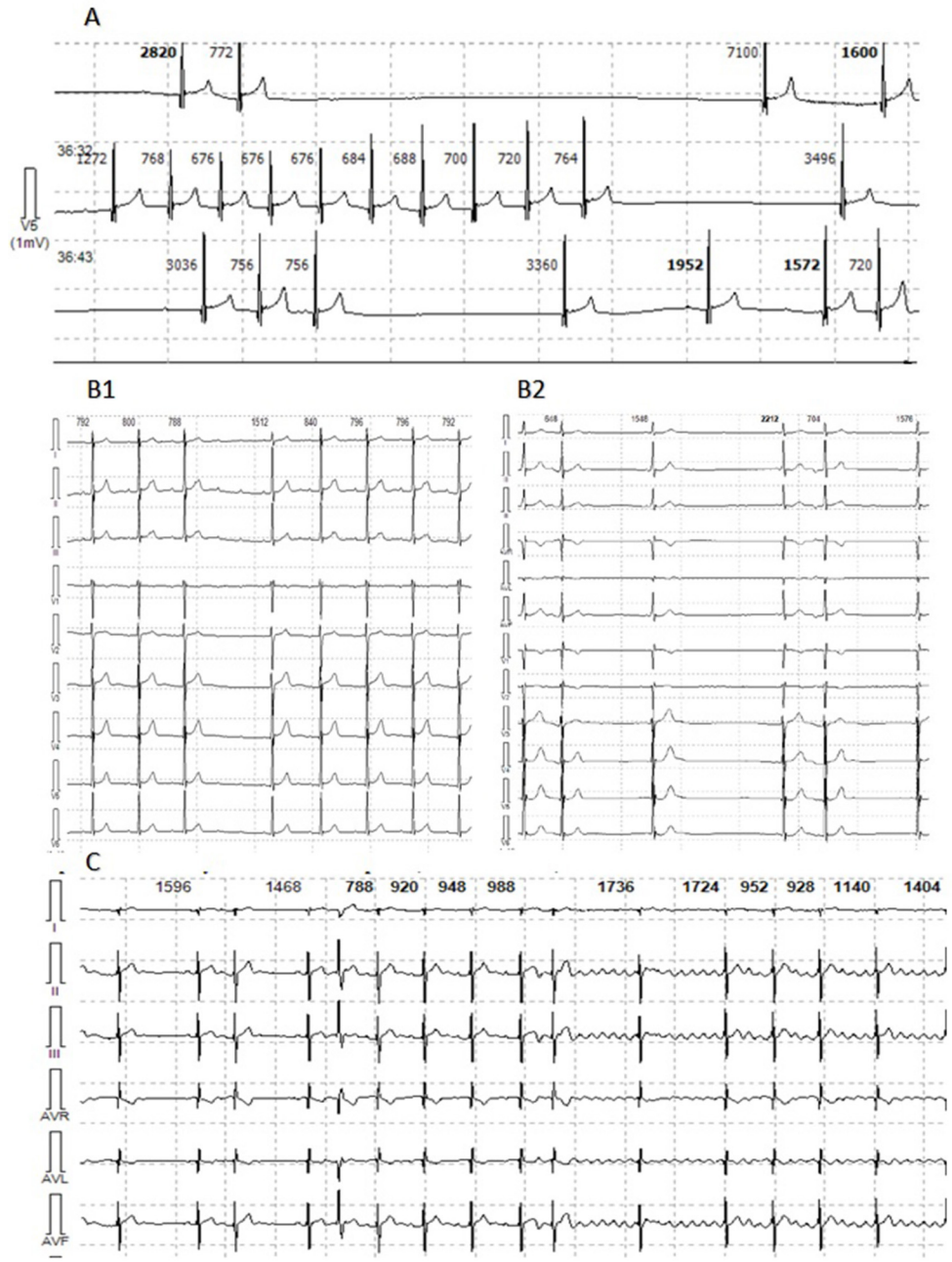

FIGURE 2 | Holter monitor fragments of Patient 1, 2, and 5. **(A)** Patient 1. Sinus arrest with pauses from 3,036 to 7,104 ms. **(B1)** Patient 2. Episode of sustained AT with variable AV conduction. **(B2)** Patient 2. Multifocal PACs. **(C)** Patient 5. Episodes of multifocal PACs, AT and AFib with variable AV conduction.

(p.Leu102Pro). Family history was unremarkable. PM was implanted along with AAT (Propafenone) at the age of 15 years.

DISCUSSION

Supraventricular tachycardia and conduction disorders with sinus and AV node dysfunction are the most common conditions in pediatric cardiology practice. The thorough diagnostic workup and precise nosology definition are important for the optimal therapeutic strategy. The presented cases as well as several earlier reports (**Table 2**) provide the evidence that EDMD has to be considered in children presenting with conduction disorders or atrial arrhythmias even without overt signs of muscular dystrophy and joint contractions. The natural cause of EDMD leads to the requirement of PM in 56% of the patients, AF/flutter developed in 61%, atria standstill in 45%, and embolic stroke

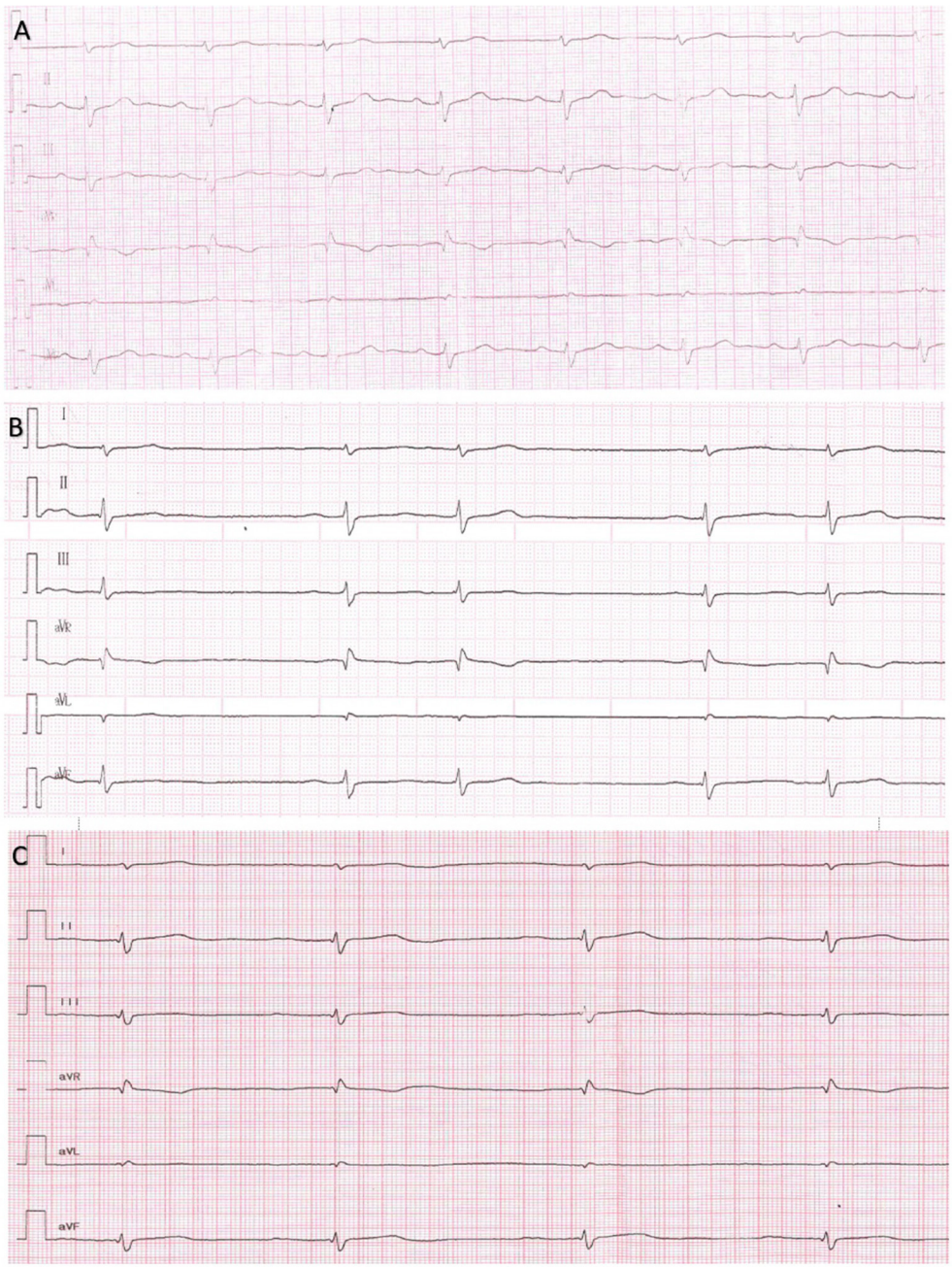

FIGURE 3 | Series of Electrocardiograms from a patient 5 showing reduced amplitude of P wave and AV conduction in dynamics. **(A)** 13 y.o. P wave amplitude 2.4 mm, P wave duration 70 ms, PQ 160 ms. **(B)** 14 y.o. P wave amplitude 1.5 mm, P wave duration 100 ms, PQ 200 ms. **(C)** 15 y.o. P wave amplitude 1 mm, P wave duration 110 ms, PQ 250 ms.

in 36% of the patients (18). Therefore, identification of EDMD-underlying genetic background is important for following genetic cascade screening first-degree relatives of patients, that is recommended and includes ECG, HM, echocardiography, and CMR (43).

The precise molecular mechanisms underlying predominantly atrial and conduction pathology in EDMD are largely unknown. Most of the genes and genetic loci identified by GWAS linked to AFib represent transcriptional factors and signaling molecules responsible for cardiac development and cardiomyocyte differentiation (44). In this context, the ability of nuclear envelope proteins to modify chromatin organization and transcriptional activity could explain the effect of *EMD* deficiency on myocardial cell fate (45, 46). A deficiency or

TABLE 2 | Literature review on heart rhythm disturbances in EDMD in childhood.

References	Year	N of patients	Gene/ Sybtype	Age of onset	Age of cardiac onset in childhood	Muscle weakness	Contractures	Cardiac involvement *in childhood
Bonne et al. (39)	2000	53 (6 families +17)	LMNA/ EDMD 2	0–40	14.7 ± 2.12 (from 11 to 17)	41 (77.3%) *40 (75.4%)	41 (77.3%)	All aged groups 41 (77.3%), incl. Isolated: 12 (29%) ***7 (13.2%),** incl. 1 isolated Arrhythmias - 5 Arr. + VD - 1 1 – n/a
Vohanka et al. (14)	2001	1	EMD/ EDMD 1	16	16	+/–	–	Atr. standstill AVB PM at 26 y.o. Afib at 27 y.o.
Sanna et al. (11)	2003	10	LMNA/ EDMD2 EDMD3	3.4 ± 1.9	14.1 ± 1.8	7 (70%)	10 (100%)	8 (80%) ***6 (60%)** AF – 2; AFib – 1; AT – 3; PACs – 2 NsVT – 3 AVB – 2; BBB – 3 SSD – 3; Atr. standstill – 1 Junct. rhythm - 1 Mild LV dil - 1
Hong et al. (40)	2005	3	EMD, LMNA/ EDMD 1 EDMD 2	1, 3, 15	14 (EDMD 1)	3 (100%)	2 (66.6%)	3 (100%)/***1** Atr. standstill, AVB, SSD, Junct.rhythm, PM, RV&RA dil
Sakata et al. (34)	2005	33 (16 carriers) *3	EMD/ EDMD 1	n/a *10, 13, 14	10, 14	*1 (6.25%)	3 (18.8%) *2	10 (62.5%) ***3 (18.8%)** SSD, AVB, PM - 1 RA dil - 2
Karst et al. (33)	2008	10 (1 family) *4	EMD/ EDMD 1	4 –teens 4 – from 28 to 31 y.o.	Teens	–	–	8 (80%) ***4** (40%) Arrhythmias – 4 Syncope - 1
Nigro et al. (41)	2010	1	EMD/ EDMD 1	5	10	+	–	SSD, Junct.rhythm, AVB, PACs, VT
Homma et al. (42)	2018	1	LMNA/ EDMD 2	3	5	+	+	AF
Fan et al. (10)	2020	84 32 – EDMD 11 - LGMD1B	LMNA/ EDMD2	EDMD: 2.2 ± 1.7 LGMD1B: 2.6 ± 3.0	indicated in column "Cardiac involvement"	17 (53.1%)	24 (75%) av. age 9.6 2 (18.2%) av. age 20.5	14 (43.8%) + 1 (9.1%) ST – 4 (av. age 6.9) + 1 (16) PACs – 1 (11) AVB – 1 (14) HF, PH, SSS – 1 (17)
Our study	2021	5	EMD, LMNA/ EDMD 1 EDMD 2	10.3 ± 4.4	13.2 ± 3.11	2 (40%)	3 (60%)	5 (100%) SVT – 3; AF – 1; AFib – 3 PACs – 4; PVCs – 1 SSD – 5; Junct.rhythm – 1 AVB – 4 PM – 2 RA dil – 1

Arr, arrhythmias; AT, atrial tachycardia; AFib, atrial fibrillation; AF, atrial flutter; AVB, atrioventricular block; BBB, bundle brunch block; HF, heart failure; mild LV dil, left ventricular dilatation; NsVT, non-sustained ventricular tachycardia; PACs, premature atrial captures; PH, pulmonary hypertension; SSD, sick sinus disfunction; ST, sinus tachycardia; VD, ventricular disfunction. Bold indicates the number of children in the study with cardiac involvement.

**Onset in childhood.*

defects of this proteins could lead to nuclear instability in tissues undergoing mechanical stress, including cardiac and skeletal muscle. Of note, another nuclear envelope protein – nesprin 2 encoded by the *SYNE2* gene and reported as a rare cause of EDMD was also identified among GWAS loci in association with AF (47, 48). In addition, Shimojima et al. reported that

mutant forms of emerin caused abnormalities in nuclear Ca^{++} transients, which may further modulate nuclear transcriptional pathways in response to mechanical stress (49). As a result, alterations in cardiomyocyte differentiation gradually led to replacing the normal myocardial cells with fibrotic tissue leading to sinus node dysfunction, ectopic loci, and conduction defects. The question remains why these processes mainly start in the atria, often involve the atrioventricular node and only eventually affect the ventricles. This question is further difficult to address because of the unavailability of atrial tissue for morphological examination and limitation of CMR for atrial imaging because of difficulties in achieving adequate image resolution in thin-walled atria (50).

Cardiac structural abnormalities, ventricular arrhythmias and dysfunction are more common in AD-EDMD and are not typical of X-linked EDMD. Of note, in the presented case series, the only patient who demonstrated rare PVCs was the Patient 4 with *LMNA*-associated EDMD. Together with other reports on *LMNA*-associated SCD in patients with cardiac and muscle pathologies, this further draws attention for the more thorough follow-up of this group in terms of different arrhythmias (51).

It is commonly accepted that cardiac symptoms of EDMD follows the neuromuscular phenotypes and becomes evident in the second-third decade of life (7, 11, 23). Cases of isolated cardiac manifestations of EDMD remain rarely reported, especially in pediatric patients (7, 33, 40). However, such cases, together with our study, emphasize the importance of genetic testing and target search for EDMD in cases of progressive refractory arrhythmias with or without specific neurological and laboratory findings. Three patients with EDMD1 we observed did not have a typical clinical course with early contractures or muscle weakness, but two patients had severe cardiac abnormalities requiring medication and intervention treatment. Patients 4 and 5 with *LMNA*-genetic variants demonstrated the clinical variability of EDMD ranging from mild disease course and later cardiac debut to early manifestation and fast-progression that led to PM and AAT. Thus, the results of genetic testing helped us draw up a plan of management and further follow-up, including annual cardiological and neurological examinations.

The main therapeutic problem we faced was coexistence of symptomatic high-frequency atrial arrhythmias and conduction disorders, which limited the prescribing of AAT. The use of RFA in EDMD is a debatable issue (52). The literature search resulted in four cases of RFA in EDMD described in details: three with poor outcomes [1 – RFA of AF with recurrence of arrhythmia, leading to systolic dysfunction and heart transplantation (53); 2 – recurrent SVT with PM after AV nodal ablation, accession of ventricular tachycardia and death from irrecoverable asystole (54); 3 – repeated unsuccessful RFA of AFib leading to embolic stroke (42)] and one successful: RFA of AF by employing a three-dimensional mapping (52). In our study, RFA was performed in two patients: successful treatment of AVNRT and AF in Patient 1 and unsuccessful RFA of mAT in Patient 2.

Since sudden death in X-linked EDMD is primarily caused by a complete heart block, it can be reliably averted by PM implantation (3). The ICD, cardiac resynchronization therapy, mechanical circulatory support, and heart transplantation remain a rare but potentially applicable strategies in EDMD patients (21, 43, 55). In the long-term longitudinal study performed by Boriani et al., heart failure requiring transplantation occurred in 6% and asymptomatic LV dysfunction in 17% of patients (18). ICD is more often required for *LMNA* mutation carriers and should be considered with sustained or non-sustained ventricular tachyarrhythmias, especially in those with LVEF $<$ 45% or in patients with indications for PM implantations (41, 56). In line with that, three of five described patients had I class indications for PM, and none had indications for ICD implantation.

CONCLUSION

In conclusion, while being rare cases, heart rhythm disorders can represent the first and for a long time, the only clinical symptom of EDMD even in the pediatric group of patients. Therefore, thorough laboratory and neurological screening along with genetic studies are of importance in each pediatric patient presenting with complex arrhythmias of primary supraventricular origin to exclude EDMD or other neuromuscular disorders. Consideration of EDMD in diagnostic workup can facilitate the optimal strategy and personalized follow-up of this group of patients.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the Gene bank repository, accession numbers SCV001548550–SCV001548554 and publicly available.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Almazov National Medical Research Centre Institute Ethical Review Boards. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

EY, SF, and TL contributed to the conception and design of the study, analysis, and interpretation of the data and drafting of the manuscript. TK contributed to the study concept and research design and wrote the manuscript. AKos, TP, and EV made contributions to the conception and design of the study and revision of the manuscript critically. VL, TV, DL, and LM took part in the analysis and interpretation of the data and have been involved in revising the manuscript critically. AR, PS, YF, AKoz, SZ, NS, and AZ conducted the experiments and performed the analysis and interpretation of the data. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.668231/full#supplementary-material

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CASE REPORT
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Case Report: Two Chinese Infants of Sengers Syndrome Caused by Mutations in *AGK* Gene

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Sengers syndrome (OMIM #212350) is a rare autosomal recessive disorder due to mutations in acylglycerol kinase (*AGK*) gene. We report two cases that were diagnosed clinically and confirmed genetically. Both infants had typical clinical features characterized by hypertrophic cardiomyopathy, bilateral cataracts, myopathy, and lactic acidosis, and heart failure was the most severe manifestation. Genetic testing of a boy revealed a homozygous pathogenic variant for Sengers syndrome in *AGK* (c.1131+2T>C) which was classified as likely pathogenic according to the ACMG guideline; besides, his skeletal muscle biopsy and transmission electron microscope presented obvious abnormity. One girl had compound heterozygous (c.409C>T and c.390G>A) variants of *AGK* gene that was identified in the proband and further Sanger sequencing indicated that the parents carried a single heterozygous mutation each. After the administration of "cocktail" therapy including coenzyme Q10, carnitine, and vitamin B complex, as well as ACEI, heart failure and myopathy of the boy were significantly improved and the condition was stable after 1-year follow-up, while the cardiomyopathy of the girl is not progressive but the plasma lactate acid increased significantly. We present the first report of two infants with Sengers syndrome diagnosed via exome sequencing in China.

Keywords: Sengers syndrome, acylglycerol kinase, mutation, genotype, cardiomyopathy, hypertrophic

INTRODUCTION

Sengers syndrome (OMIM #212350) is a rare autosomal recessive disorder due to mutations in the acylglycerol kinase (*AGK*) gene (1). The disease was first described by Sengers in 1975 with the hallmark signs of hypertrophic cardiomyopathy, congenital cataract, mitochondrial myopathy, and lactic acidosis after exercise and can be further divided into two clinical forms including a severe neonatal form that can cause infantile death and a benign form with better prognosis (1, 2). Nystagmus, esotropia, eosinophilia, and cervical meningocele are relatively rare clinical manifestations (3, 4).

The *AGK* gene is located on chromosome 7q34 and consists of 16 exons (1). To date, several studies have identified different types of loss-of-function mutations in the *AGK* gene,

including start codon mutations, nonsense, frameshift, and splice site mutations (1–3, 5–16). AGK is a mitochondrial protein that catalyzes the phosphorylation of diacylglycerol (DAG) and monoacylglycerol (MAG) to phosphatidic acid (PA) and lysophosphatidic acid (LPA), respectively. PA feeds into the synthesis of the mitochondrion-specific lipid cardiolipin (CL), which is essential for mitochondrial structure and function (2).

Sengers syndrome is often misdiagnosed due to its rarity. Currently, <40 cases diagnosed by genetic testing have been reported in the literature although other cases may not have been reported and the prevalence remained unclear. There is no cure for this disease and its clinical features and prognosis are also still unclear. We present the clinical characteristics and molecular basis of Sengers syndrome in two Chinese children, the first reported cases in China.

CASE PRESENTATION

Patient 1

This infant boy was the second child of healthy consanguineous parents from China. After an uneventful pregnancy, he was born at term by vaginal delivery, with a birth weight of 3,260 g, and 10 years after the first sibling. The Apgar score was 9, 10, 10 at 1, 5, 10 min, respectively. The boy was healthy during the first 2 months of life, and he had a mild motor developmental delay characterized by a disability of sitting without support at 7 months of age. His cognitive condition was normal. Bilateral total cataracts were noticed when he was 1 month old. Cataract phacoemulsification and vitrectomy of both eyes were performed at 3 months of age and aphakia was corrected with glasses and intraocular lens implantation was scheduled. The boy was coughing and had inspiratory stridor for 6 days at the age of 8 months of age and was admitted to our hospital. Physical examination showed no obvious dysmorphic features, no rash, shortness of breath, a rapid heart rate of 180 bpm, no heart murmur, and moderate hepatomegaly. Moreover, severe hypotonia and decreased muscle strength were observed (grade 2 of MRC 6-point scale). The chest X-ray showed an enlarged heart, the cardiothoracic ratio (CTR) reached about 0.65 (**Figure 1a**). Echocardiography showed hypertrophic cardiomyopathy with a maximum septal thickness of 8 mm, a posterior wall thickness of 8 mm, no outflow tract obstruction, enlargement of left ventricle, and ejection fraction of 39% (**Figure 1b**). Electrocardiogram showed sinus tachycardia, short PR interval (100 ms), biventricular hypertrophy, and multi-lead ST-segment changes (**Figure 1c**). Arterial blood gas results were relatively normal except for mild lactic acidosis (lactate 3.7 mmol/L, reference ranges: lactate <2.0 mmol/L). Other laboratory analyses were normal which included blood routine testing, routine urine testing, alanine transaminase, aspartate aminotransferase, creatinine, urea nitrogen, blood glucose, and blood ammonia levels. N-terminal pro-brain natriuretic peptide (NT-proBNP) reached 6,076 pg/ml (reference ranges <125 pg/ml) reflecting the state of the heart failure. Further metabolic work-up, such as urinary organic acids and amino acids, blood carnitine, and acid alpha-glucosidase tests were also normal.

The medical exome of the proband was enriched before sequencing which included about 4,200 known disease genes as well as known pathogenic variants located in deep intronic and other non-coding regions. After genetic analysis, a novel homozygous (c.1131+2T>C) variant of *AGK* gene was identified in the proband. Both parents were unaffected and further Sanger sequencing indicated that both the parents were heterozygous for this change (**Figure 2**). The c.1131+2T>C variant in the *AGK* gene has not been previously reported in clinical cases. However, defects in *AGK* gene have been reported to be associated with Sengers syndrome. The clinical presentation includes congenital cataracts, hypertrophic cardiomyopathy, skeletal myopathy, exercise intolerance, and lactic acidosis. Mental development is normal, but affected individuals may die early from cardiomyopathy (2). Skeletal muscle biopsies of two affected individuals showed severe mtDNA depletion (8). Altogether, *AGK* gene was identified as the causative gene in the proband and the variant c.1131+2T>C was classified as likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

A muscle biopsy was performed revealing obvious structural changes. HE stain showed that the muscle fibers were slightly different in size, and the small fibers were mostly small round and polygonal in shape (**Figure 3a**). Cavitation and fissures were observed in the fibers. Moreover, most fibers had deeply stained sarcolemma. Modified Gomeri tricolor (MGT) stain displayed a large number of muscle fibers showing cytoplasmic and sub-sarcolemma vacuole fissures with RRF-like changes (**Figure 3b**). Deep staining was observed for sarcolemma in many fibers with nicotinamide adenine dinucleotide (NAD), succinate dehydrogenase (SDH), and cytochrome *c* oxidase (COX) staining (**Figures 3c–e**). Furthermore, the content of glycogen of a small number of fibers increased significantly with periodic acid Schiff (PAS) staining and the content of lipid droplets in a large number of fibers markedly increased in oil red (ORO) staining (**Figure 3f**). Transmission electron microscope (TEM) revealed an increased number of lipid droplets in many fibers and the mitochondria were abnormal with a severe loss of cristae (**Figures 3g,h**).

Supplementation with coenzyme Q10, carnitine, B-vitamins, and biotin (called mitochondrial cocktail) was given daily, associated with angiotensin converting enzyme (ACE) inhibitors for cardiomyopathy management. The patient received milrinone, diuretics (furosemide and spironolactone), and captopril to improve heart function. Levocarnitine (100 mg/kg daily), coenzyme Q10 (1 mg/kg daily), and vitamin B complex (vitamin B_1 20 mg/day and riboflavin 10 mg/day) were administered to improve metabolic status; however, anti-infection and other symptomatic treatments were also applied. After 10 days of treatment, respiratory symptoms were alleviated, and muscle strength improved to grade 5 of MRC 6-point scale. Echocardiography showed no significant improvement in myocardial hypertrophy(septal thickness of 9 mm, a posterior wall thickness of 10 mm), left ventricular end-diastolic dimension (LVEDd) reduced from 33 to 23 mm, and heart function returned to normal (EF of 67%). Contrary to clinical improvement, plasma lactate acid rose to 13 mmol/L. Milrinone was stopped and the remaining oral drugs continued to be used. To date, this child has been followed up for 8 months and is 18 months of age. He had no recurrent respiratory

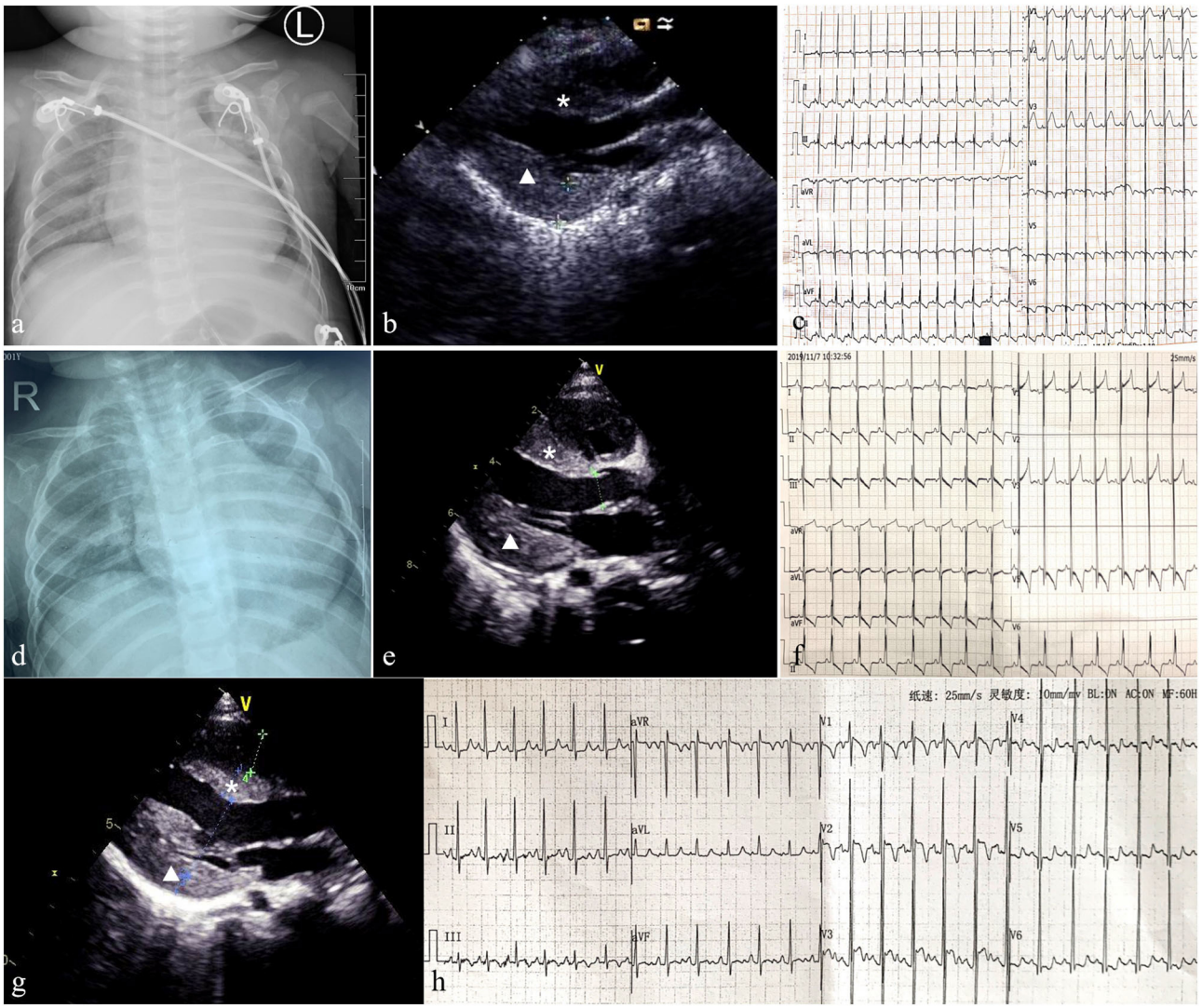

FIGURE 1 | Patient 1: Chest X-ray showed an enlarged heart (the cardiothoracic ratio reached about 0.65) **(a)** and echocardiography indicated hypertrophic non-obstructive cardiomyopathy **(b)**. ECG showed sinus tachycardia, short PR interval, biventricular hypertrophy, and multi-lead ST segment changes **(c)**. Follow-up for 8 months, chest X-ray indicated the CTR was 0.65 **(d)**. Echocardiography showed the thickness of IVS and LVPW were 9 and 11 mm, respectively, with no outflow obstruction, and LVEF was 60% **(e)**. ECG revealed multiple lead ST-T changes and left ventricular hypertrophy **(f)**. Patient 2: Echocardiography showed the thickness of interventricular septum (IVS) and left ventricular posterior wall (LVPW) were 9 and 10 mm, respectively **(g)**. ECG revealed left ventricular hypertrophy and multiple lead ST-T changes **(h)**. The asterisk represents IVS and triangle represents LVPW.

infections, his height and body weight were 75 cm and 8 kg, respectively, and no cognition delay was detected. Mild motor retardation existed which was characterized by walking utilizing support. Physical examination showed no tachypnea, an average heart rate of 100 bpm, lack of cardiac positive signs, no liver enlargement, no hypotonia, and muscle strength grade 5. Plasma lactate acid was 3.1 mmol/L. The chest X-ray indicated that the CTR was 0.65 (**Figure 1d**). Echocardiography showed a septal thickness of 9 mm, a posterior wall thickness of 11 mm, and LVEF of 66% (**Figure 1e**). ECG revealed multiple lead ST-T changes and left ventricular hypertrophy (**Figure 1f**).

Patient 2

A girl was the third child born by normal delivery at full term after a normal pregnancy with a birth weight of 2250 g. His older sister died of "brain herniation" at 4 months old, and his older brother is alive and healthy. The newborn had no obvious muscle hypotonia. At age 2 months, bilateral cataracts were noted and cataract phacoemulsification was performed at 3 months of age. In the following 3 months, the patient developed growth retardation; physical examination showed no obvious dysmorphic features, no heart murmur, no hepatomegaly, no hypotonia, and decreased muscle strength was observed (grade 4 of MRC 6-point scale). HCM was detected that echocardiography

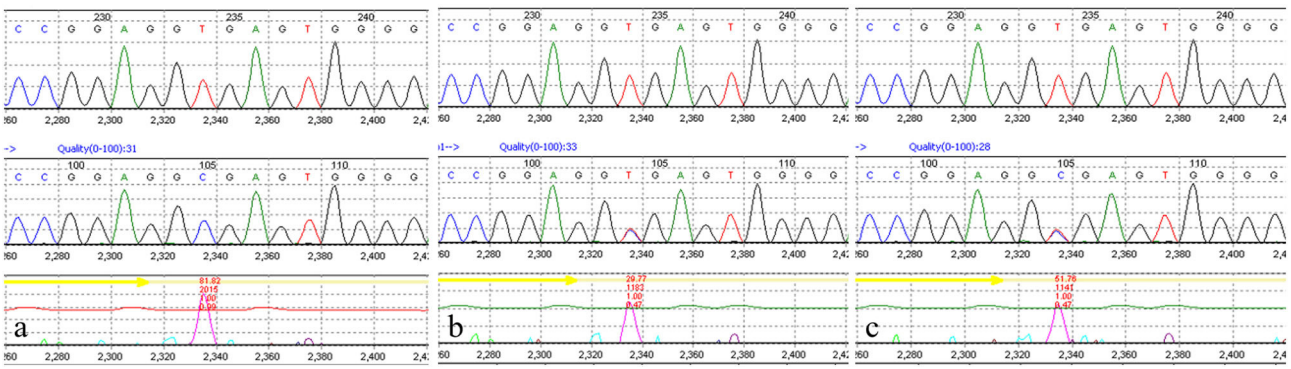

FIGURE 2 | Molecular study of the genomic DNA of patient 1 detected a novel AGK homozygous mutation (c.1131 + 2T>C) in the proband **(a)**. Both parents were confirmed to be heterozygous carriers **(b,c)**.

revealed a septal thickness of 9.1 mm and posterior wall thickness of 9.5 mm without the presence of an obstructive component, and electromyographic activity was weak. Blood gas analysis revealed metabolic acidosis with elevated serum lactic acid (4.1 mmol/L, normal <2.0 mmol/L). Urinary organic acid analysis showed increased amounts of 3-hydroxybutyrate (25.5 mmol/L, normal <9.0 mmol/L) and NT-proBNP reached 253 pg/ml. Laboratory studies in serum yielded normal results for the following: hematological parameters, electrolytes, liver function, renal function, creatine kinase, cholesterol, triglycerides, glucose, and ammonia levels.

The medical exome of the proband was used for genetic analysis, compound heterozygous (c.409C>T and c.390G>A) variants of *AGK* gene were identified in the proband, and further Sanger sequencing indicated that the parents carried a single heterozygous mutation each. The nonsense c.409C>T variant in the *AGK* gene has already been described at a homozygous state in several cases which had typical Sengers syndrome manifestations. The splicing c.390G>A variant in the *AGK* gene has not been previously reported in clinical cases and was classified as likely pathogenic according to the ACMG guidelines by genetic evaluation of pathogenicity of variants using multiple computational algorithms. Currently, this patient has been treated with "mitochondrial cocktail," ACEI, and beta-blockers for more than 1 year. She has significant growth retardation and moderate muscle weakness, especially in the lower limb muscles, and she can just keep a standing position with help. The good part is that the cardiomyopathy is not progressive which echocardiography showed a septal thickness of 9 mm and posterior wall thickness of 10 mm (**Figure 1g**). ECG revealed left ventricular hypertrophy and multiple lead ST-T changes (**Figure 1h**). However, the serum lactic acid increased significantly, reaching 14.99 mmol/L, so appropriate limitation of physical activities is recommended in daily life.

DISCUSSION

Sengers syndrome is a rare mitochondrial disease caused by mutations in the *AGK* gene (4). To date, the incidence of this disease is difficult to estimate and the cases in our study are the first cases reported in China. AGK, also known as multi-substrate lipid kinase (MULK), affects the synthesis of phosphatidic acid which acts as a second messenger regulating a number of cellular processes and plays an important role in the synthesis of phospholipids (1). Many studies found oxidative phosphorylation (OXPHOS) defects in Sengers syndrome and suggested that mitochondrial respiration and metabolism are affected in the absence of AGK (1, 3, 7). Kang et al. suggested that AGK is a subunit of the mitochondrial TIM22 protein import complex where it facilitates the import and assembly of mitochondrial carrier proteins. Furthermore, the TIM22 complex and carrier import was demonstrated to be affected in Sengers syndrome cells and tissues (5). These findings showed that the role of AGK in Sengers syndrome patients may explain the characteristics of mitochondrial morphology, cataracts, and respiratory chain dysfunction.

Before the widespread application of genetic testing and the determination of pathogenic mutations in *AGK* gene, the diagnosis of Sengers syndrome mainly relied on characteristic clinical manifestations. Therefore, some studies have reported some confirmed or suspicious cases without molecular diagnostic evidence (17–19). Herein, we reviewed previous studies on this syndrome and summarize the characteristics of the cases with both clinical and genetic information (**Table 1**, **Supplementary Material 1**). Thus far, a total of 38 children have been diagnosed with the confirmation of gene test, of which 60% were males. In terms of mortality, Sengers syndrome is a highly malignant disease, with a total mortality rate of 57.9% (22/38). Importantly, the death rate varied at different ages, namely, 86.4% (19/22) within 3 years of age, 77.3% (17/22) within 1 year of age, and 31.8% (7/22) during the neonatal period. Concerning the clinical manifestations, the overwhelming majority (26/38, 68.4%) of the patients had their features in the neonatal period. Cataracts were the most common clinical manifestation (94.7%), followed by hypertrophic cardiomyopathy (65.8%), lactic acidemia (71.1%), and myopathy (65.8%). Other rare clinical manifestations included nervous system issues, such as cerebellar non-hemorrhagic stroke, and ocular signs, such as

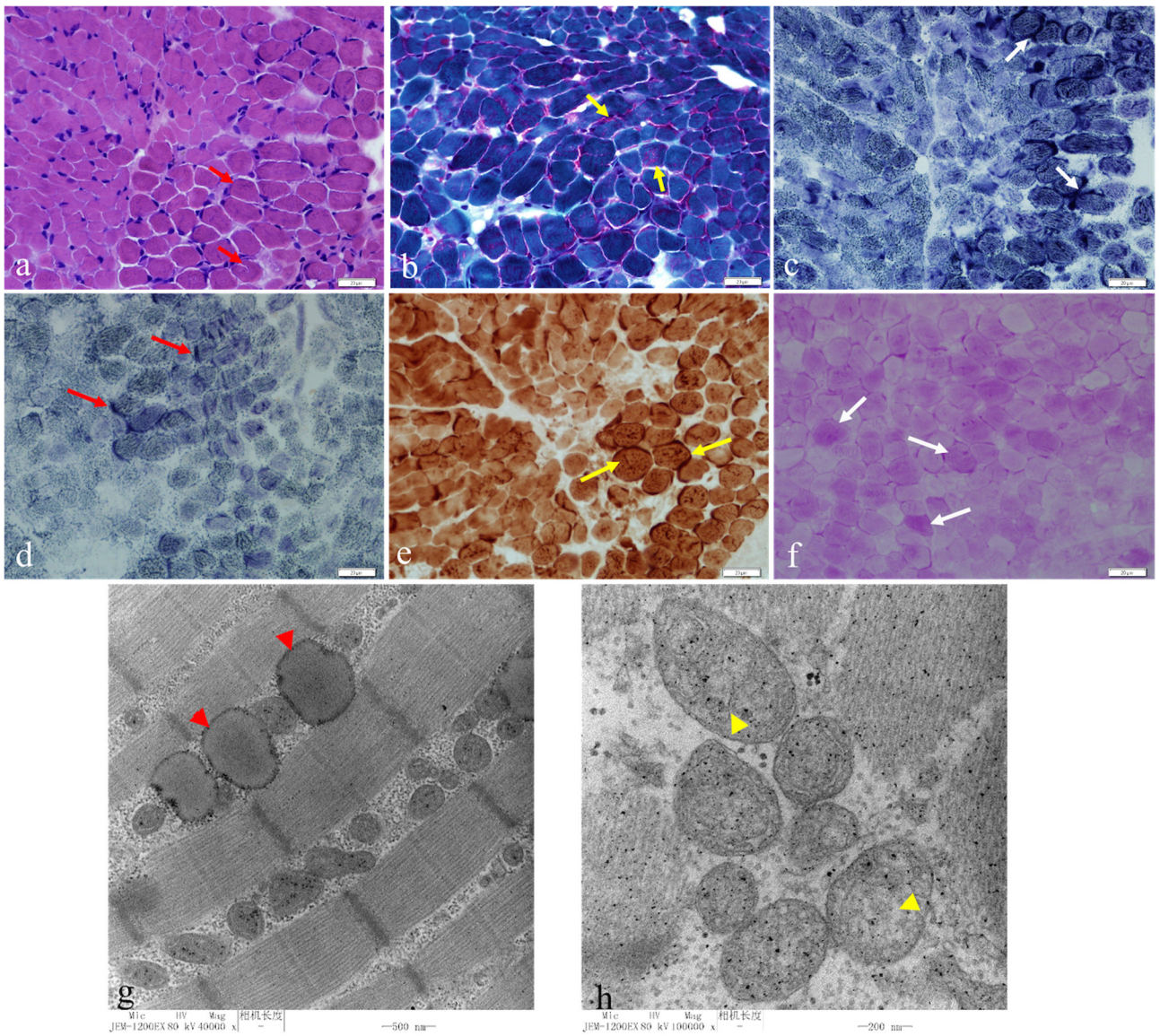

FIGURE 3 | HE staining showed that the muscle fibers were slightly different in size, and the small fibers were mostly small round and polygonal in shape. Cavitation and fissures were observed in the fibers with most fibers deeply staining the sarcolemma **(a)** (red short arrow, bar 20 μm). Modified Gomeri tricolor (MGT) staining displayed a large number of muscle fibers showing cytoplasmic and sub-sarcolemma vacuole fissures with RRF-like changes **(b)** (yellow short arrow, bar 20 μm). Deep staining was seen under the sarcolemma in many fibers in nicotinamide adenine dinucleotide (NAD) **(c)** (white short arrow, bar 20 μm), succinate dehydrogenase (SDH) **(d)** (red long arrow, bar 20 μm), cytochrome *c* oxidase (COX) staining **(e)** (yellow long arrow, bar 20 μm). The glycogen content of a small number fibers increased significantly with periodic acid Schiff (PAS) staining **(f)** (white long arrow, bar 20 μm). Transmission electron microscopy (TEM) showed an increased number of lipid droplets in many fibers **(g)** (red arrowhead, bar 500 nm) and the mitochondria were abnormal with a severe loss of cristae **(h)** (yellow arrowhead, bar 200 nm).

nystagmus. The most fatal features were cardiac failure and cardiac arrest.

Considering the typical clinical manifestations of Sengers syndrome, we need to differentiate it from syndromes characterized by hypertrophic cardiomyopathy, muscle weakness, and growth retardation, such as RASopathy disorder and Pompe disease (20, 21). These syndromes often have hypertrophic cardiomyopathy as the primary clinical feature, and muscle weakness can also be easily found through physical examination. In particular, we should note that in the case of combined infection, worsening heart failure, etc., severe hyperlactatemia can also occur in the aforementioned diseases. Certainly, there are also some special points of differential diagnosis. Patients with RASopathy disorder often have more typical facial abnormalities, and congenital heart diseases, such as pulmonary valve stenosis and ventricular septal defect, are more common (20). Pompe disease and other inherited metabolic diseases often have a definite enzymatic deficiency, and the test

TABLE 1 | Clinical and molecular findings in patients with Sengers-syndrome caused by AGK mutations.

Case	Described by	Gender	Mutations		Onset of disease	Cardiomyopathy	Cataract	Myopathy	LA	Other presentation	Biopsy	OXPHOS defect	Status
			Allele 1	Allele 2									
1	Present case 1	M	c.1131+2T>C, Splicing defect	N	8m	+	+	+	+	–	+	NA	Alive 2 y
2	Present case 2	F	c.409C>T, p.Arg137*	c.390G>A, p.Glu130Glu	2m	+	+	+	+	–	–	NA	Alive 2.5 y
3	Elliott et al. (9)	F	c.409C>T, p.Arg137*	c.841C>T, p.Arg281*	1d	+	+	NA	NA		NA	NA	NA
4	Lalive et al. (10)	M	c.3G>C, p.Met1Ile	c.517C>T, p.Gln173*	3m	+	+	+	+		NA	NA	Alive 6 y
5	Lalive et al. (10)	M	c.3G>C, p.Met1Ile	c.672C>A, p.Tyr224*	3m	+	+	+	+		NA	NA	Alive 35 y
6	Morava et al. (4)	M	c.1131+5G>A, Splicing defect	N	18m	+	+	–	+		+	I, II + III, IV, V, PDHc	Dead 12 y
7	Morava et al. (4)	F	c.1131+5G>A, Splicing defect	N	5m	+	+	+	+		+	I, II + III, IV, V, PDHc	Alive 10 y
8	Van Ekeren et al. (12)	F	c.1131+5G>A, Splicing defect	N	Birth	+	+	+	+	Stroke, cerebrovascular accident		V	Alive 41 y
9	Rosa et al. (12)	F	c.221+1G>A, Splicing defect	c.1213C>T, p.Gln405*	Birth	+	+	+	+		+	–	Alive 12 y
10	Mayr et al. (2)	M	c.306C>T, p.Tyr102*	c.841C>T, p.Arg281*	Birth	+	+	–	+	Floppy infant	+	I, II+III, IV, V	Dead 18 d
11	Mayr et al. (2)	F	c.672C>A, p.Tyr224*	c.870del, p.Gln291Argfs*8	Birth	+	+	+	+	Cardiac arrest	+		Dead 10 m
12	Mayr et al. (2)	M	c.101+?222–?del	N	4m	+	+	+	+	Seizures, upper left limb paresis, brain ventricles dilatation	+		Dead 8 m
13	Mayr et al. (2)	M	c.412C>T, p.Arg138*	c.1137_1143del, p.Gly380Leufs*16	1w	+	+	+	+		+	I	Dead 11 m
14	Haghighi et al. (1)	M	c.523_524del#p.Ile175Tyrfs*2	N	1m	+	+	+	+	Nystagmus, floppy infant	NA	NA	Dead 7 m
15	Haghighi et al. (1)	M	c.424-1G>A, Splicing defect	N	Birth	+	+	–	+		NA	NA	Dead 10 d
16	Haghighi et al. (1)	F	c.424-1G>A, Splicing defect	N	Birth	+	+	–	+	Eosinophilia	NA	NA	Dead 4 m
17	Haghighi et al. (1)	F	c.409C>T, p.Arg137*	N	NA	+	+	–	+	Esotropia	Fatty infiltrations (heart)	I	Dead 3 m
18	Haghighi et al. (1)	M	c.409C>T, p.Arg137*	N	Birth	+	+	+	+	Esotropia, nystagmus, floppy infant	+	I	Dead 6 m
19	Haghighi et al. (1)	M	c.871C>T, p.Gln291*	c.1035dup, p.Ile346Tyrfs*39	Birth	+	+	+	+			NA	Alive 3 m

(Continued)

TABLE 1 | Continued

Case	Described by	Gender	Mutations		Onset of disease	Cardiomyopathy	Cataract	Myopathy	LA	Other presentation	Biopsy	OXPHOS defect	Status
			Allele 1	Allele 2									
20	Haghighi et al. (1)	F	c.297+2T>C, p.Lys75Glnfs*12	c.841C>T, p.Arg281*	Birth	+	+	+	−	Cervical meningocele, language delay		I	Alive 10 y
21	Haghighi et al. (1)	M	c.877+3G>T, Splicing defect	N	Birth	+	+	+	−			NA	Alive 15 y
22	Calvo et al. (8)	F	c.297+2T>C, p.Lys75Glnfs*12	c.1170T>A, p.Tyr390*	<1y	+	+	+	+	Headaches, osteopenia, premature ovarian failure	+	I, III, IV	Dead 18 y
23	Calvo et al. (8)	F	c.1131+1G>T, p.Ser350Glufs*19	N	Birth	+	+	−	+		+	I, III, IV	Dead 4 d
24	Siriwardena et al. (3)	F	c.979A>T, p.Lys327*	N	Birth	+	+	+	NA	Cardiac failure		NA	Dead 5 m
25	Siriwardena et al. (3)	F	c.979A>T, p.Lys327*		Birth	+	+	−	−	Upper respiratory tract infection		I, I + III, II + III, III, IV, high CS	Dead 12 d
26	Siriwardena et al. (3)	M	c.979A>T, p.Lys327*	N	Birth	NA	NA	NA	NA		NA	NA	Dead 2 d
27	Siriwardena et al. (3)	M	c.979A>T, p.Lys327*	N	Birth	NA	NA	NA	NA		NA	NA	Dead 18 d
28	Siriwardena et al. (3)	M	c.3G>A, p.Met1?(p.M1I)	N	9m	+	+	+	+	Cerebellar non-hemorrhagic stroke, cardiac arrest, ventricular fibrillation	Scattered COX negative fibers	NA	Dead 15 m
29	Siriwardena et al. (3)	M	c.3G>A, p.Met1?(p.M1I)	N	Birth	+	+	+	+	Cerebellar non-hemorrhagic stroke	−	NA	Alive 2.5 y
30	Aldahmesh et al. (7)	F	c.424-3C>G, p.Ala142Thrfs*4	N	Birth	−	+	−	−		NA	NA	Alive 17 y
31	Aldahmesh et al. (7)	M	c.424-3C>G, p.Ala142Thrfs*4	N	Birth	−	+	−	−		NA	NA	Alive 11 y
32	Aldahmesh et al. (7)	M	c.424-3C>G, p.Ala142Thrfs*4	N	Birth	−	+	−	−		NA	NA	Alive 7 y
33	Kor et al. (13)	M	c.297G>T, p.K99N	N	5d	+	+	+	+	Cardiac failure	Fatty infiltrations	NA	Dead 22 m
34	Kor et al. (13)	F	c.412C>T, p.R138*	N	1m	+	+	+	+	Cardiac failure	NA	NA	Dead 3 m

(Continued)

TABLE 1 | Continued

Case	Described by	Gender	Mutations		Onset of disease	Cardiomyopathy	Cataract	Myopathy	LA	Other presentation	Biopsy	OXPHOS defect	Status
			Allele 1	Allele 2									
35	Allali et al. (14)	M	c.1035dup, p.Ile346Tyrfs*39	N	3m	+	+	+	+	Macrocephaly, cognitive deficiency, nystagmus	NA	NA	Alive 9 y
36	Allali et al. (14)	M	c.1035dup, p.Ile346Tyrfs*39	N	Birth	+	+	+	NA	Phenylketonuria, nystagmus, language delay	NA	NA	Dead 2 y
37	Beck et al. (15)	M	c.979A >T, p.K327*	N	Birth	+	+	+	+	Chorioamniotis, hepatic dysfunction	NA	NA	Dead 1 d
38	Guleray et al. (16)	F	c.1215dupG, p.Phe406Valfs4	N	3m	+	+	+	+	–	Lipid deposition Decreased COX Staining (heart and liver)	NA	Dead 9 m

M, male; F, female; N, none; NA, not available; LA, lactic acidosis; OXPHOS, oxidative phosphorylation; +, presence of condition; –, absence of condition; unknown when empty; I, complex I; II + III, succinate cytochrome c oxidoreductase; IV, cytochrome c oxidase; V, oligomycin-sensitive ATPase; PDHc, pyruvate dehydrogenase complex; d, day; m, month; y, year.

methods are efficient and convenient to facilitate early diagnosis. The main point of distinguishing Sengers syndrome from the above diseases is ocular lesions. However, due to the early onset of Sengers syndrome, the condition of ocular involvement may be missed, resulting in misdiagnosis or delay in diagnosis.

Given that Sengers syndrome is a very rare genetic disorder, the genotype/phenotype correlation has remained unclear. The severity of the disease is dictated by the combination of the two alleles, and so a child homozygous for a more deleterious mutation (e.g., a nonsense mutation) or compound heterozygous for two severe deleterious mutations may be expected to have earlier mortality; a child homozygous for a less deleterious mutation may have longer survival. This result is consistent with previous reports that homozygous *AGK* nonsense mutations have resulted in a severe form of Sengers syndrome (1). Aldahmesh et al. identified a splice site mutation causing isolated congenital cataracts in three patients, and it can be speculated that a small proportion of the normally spliced transcript can still be formed (1, 7).

Genetic investigations confirmed that one of our patients has Sengers syndrome due to a novel homozygous variant in the *AGK* gene which the splicing algorithm (splice-port) predicted to affect the splice donor site of intron 15 (c.1131+2T>C). This site is highly conserved across species and this mutation has not been reported in clinical cases. Moreover, other software programs predict that it may affect splicing. Combined with the typical clinical manifestations, pathological changes of the child, we classified this variant as pathogenic or mutation. Three individuals from two more families from the Netherlands harbored homozygous mutations near the c.1131 site which the splice-port algorithm predicted to affect the splice donor site of intron 16 (c.1131+5G>A) (2). Cardiomyopathy, cataract, and lactic acidosis were common manifestations in all three patients, which is consistent with our study. OXPHOS defects were detected in two patients; only one patient died (death at 12 years old), and the remaining two patients were reported to be alive at the time of the report (42 and 10 years of age) (2). Overall, these patients had a relatively long lifespan, and this may suggest a better prognosis for intron splicing mutations near c.1131 area. The nonsense c.409C>T variant in the *AGK* gene has already been described at a homozygous state in several typical Sengers syndrome cases, and this suggests that the gene is pathogenic (1). According to the clinical manifestations and the results of genetic analysis, the splicing c.390G>A variant is likely to be pathogenic, and further evidence needs to be accumulated. However, the clinical manifestations are different among the reported cases. It may be plausible that the characteristics may be associated with genotype. For instance, *AGK* homozygous nonsense mutations are common in critical patients which can develop cardiomyopathy and fatal lactic acidosis in infancy (1, 2, 4).

There is currently no curative therapy for mitochondrial disorders, although symptomatic measures can be highly effective and greatly improve the quality of life and outcomes for these patients (22). According to current investigations and the small population with Sengers syndrome, there is no specific treatment strategy for this disease. In our study, following the administration of anti-heart failure drugs and "cocktail" therapy, the boy's heart failure, myopathy, and other clinical features significantly improved and the girl's cardiomyopathy is not progressive. This result suggested that symptomatic treatment has a significant effect on this disease, and the clinical outcome from cardiac side is likely related to anti-failure drugs. Treatment for energy metabolism may be also an effective strategy for ameliorating respiratory chain disorders; however, it should also be noted that the "cocktail" treatment is not backed up by evidence yet, although common in clinical practice and unlikely harmful. In addition, drug therapy may not be a need for the lactic acidosis seen in Sengers syndrome, and limiting the amount of exercise may have a role in the controlling of hyperlactatemia.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/clinvar, VCV000987519; VCV000987518; VCV000209130.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of Qingdao Women and Children's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BW was responsible for interpretation of the data, drafting of the article, and approval of the final version to be published. ZD was responsible for interpretation of the data and drafting of the article. GS was responsible for data collection. CY's laboratory was responsible for specimen processing. ZL was responsible for the study conception, interpretation, revision of the article, and approval of the final version to be published. All authors read and approved the final article.

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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.2021.639687/full#supplementary-material

Supplementary Material 1 | Gene structure of AGK and localization of identified mutations (1–3, 5–16). Red font indicates newly reported mutations.

Supplementary Material 2 | Normal range for reported laboratory values.

Supplementary Material 3 | Sequential echocardiogram results of two cases.

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REVIEW
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Childhood Hypertrophic Cardiomyopathy: A Disease of the Cardiac Sarcomere

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Hypertrophic cardiomyopathy is the second most common cause of cardiomyopathy presenting during childhood and whilst its underlying aetiology is variable, the majority of disease is caused by sarcomeric protein gene variants. Sarcomeric disease can present at any age with highly variable disease phenotype, progression and outcomes. The majority have good childhood-outcomes with reported 5-year survival rates above 80%. However, childhood onset disease is associated with considerable life-long morbidity and mortality, including a higher SCD rate during childhood than seen in adults. Management is currently focused on relieving symptoms and preventing disease-related complications, but the possibility of future disease-modifying therapies offers an exciting opportunity to modulate disease expression and outcomes in these young patients.

Keywords: paediatric, hypertrophic cardiomyopathy, sarcomere, sudden death, progression

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The prevalence of childhood Hypertrophic Cardiomyopathy (HCM) is estimated to be ∼3 in 100,000 (1) live births with an annual incidence between 0.24 and 0.47/100,000 (1–3), making it the second most common cardiomyopathy presenting during childhood. The underlying aetiology is more heterogeneous than seen in adult populations and includes inborn errors of metabolism, RASopathy syndromes and neuromuscular disease. Historically, it was believed that sarcomeric disease was uncommon during childhood but it is now recognised that in most children, HCM is caused by sarcomere protein gene variants. This article provides a review of current knowledge of sarcomeric childhood HCM, highlighting the variability in disease expression and unanswered questions.

GENETICS OF SARCOMERIC HCM IN CHILDHOOD

Up to two thirds of children with non-syndromic HCM have a disease-causing variant in a sarcomere protein gene identified on genetic testing (4–6), which is usually inherited as an autosomal dominant trait. A sarcomeric variant is less likely to be identified in those presenting in infancy (approximately one third) but the overall yield of genetic testing in childhood is similar to, or even higher than, reported in adult cohorts (7). The majority of variants (70–80%) are found in β-myosin heavy chain (MYH7) or myosin-binding protein C (MYBPC3) with the proportion of disease attributable to MYBPC3 increasing with age. A smaller number of patients have variants in other sarcomeric proteins (4, 6, 8) as described in **Table 1**. Homozygosity or compound heterozygosity has been associated with early onset disease, severe phenotypes and poor clinical outcomes but is uncommon (<5%) even in childhood cohorts (18, 19, 21). The majority of childhood onset disease is therefore caused by single disease-causing sarcomeric variants. Small paediatric studies have reported that predictors of finding a disease-causing variant in childhood patients include a family history of HCM, pattern and degree of hypertrophy (22).

TABLE 1 | Reported genotype-phenotype correlations in hypertrophic cardiomyopathy.

Disease causing variant	%	Gene	Reported genotype-phenotype associations
Thick filament	75–80%	Myosin Binding Protein C3(MYBPC3)	Disease expression heterogeneous, marked age related penetrance (late onset disease common). (9, 10) Founder mutations seen (11).
		Myosin Heavy Chain 7 (MYH7)	Variants in the converter region of MYH7 associated with worse prognosis (12). Restrictive phenotypes reported (13, 14)
Thin filament	15–20%	Troponin T (TNNT2)	Minimal hypertrophy (15). Right atrial dilatation (13). Small studies reported an increased arrhythmic risk but this has not been replicated (15, 16).
		Troponin I (TNNI3)	Restrictive phenotype (14)
		Alpha-cardiac actin (ACTC)	Apical hypertrophy and LVNC (17)
		Essential myosin light chain (MYL3), Regulatory myosin light chain (MYL2), Alpha-tropomyosin (TPM1), Cardiac troponin C (TNNC1), Alpha myosin heavy chain (MYH6)	
Other	<1	*Z* disk proteins (e.g., CSPR3), phospholamban	
Compound heterozygosity/homozygosity	<5%	MYBPC3	Early onset, severe hypertrophy and poor outcome (18–20)

Familial sarcomeric HCM is associated with age-related and variable penetrance (23). Whilst some individuals will develop hypertrophy in early childhood, other mutation carriers may never develop significant features of disease (23). Disease phenotype, progression, and outcomes are likewise highly variable, even amongst family members carrying an identical mutation (9). Childhood-onset disease is more likely if there is a family history of early-onset disease (24) but as yet unidentified genetic and epigenetic modifiers are likely to play an important role in the expression of primary disease-causing sarcomeric mutations. Recent genome wide association studies have described the importance of common genetic variants on the risk of developing a HCM phenotype although the contribution of additional variants to childhood phenotypes has not yet been described (25, 26).

PRESENTATIONS AND SYMPTOMS

The underlying aetiology is at least partly responsible for determining the age of presentation with childhood HCM and patients with underlying metabolic or syndromic disease are more likely to present in infancy or early childhood (27–29). While sarcomeric HCM has historically been described to be a disease of adolescence or early adulthood, recent European and North American population cohort studies have shown a peak in presentation during adolescence and also describe presentation throughout the childhood years, including an important minority (∼14%) in the first year of life (6, 29). Such studies highlight that sarcomeric disease can present at any age, including the very young.

A diagnosis of HCM in childhood may be made following referral for symptoms (such as chest pain, palpitations, syncope, or dyspnoea), ECG abnormalities or through family screening (24, 27, 30, 31). A small proportion (3–4%) are diagnosed following a resuscitated cardiac arrest (1, 32). Infants have been described as being more likely to present with symptoms, but this may reflect the inclusion of patients with syndromic disease in population studies who are recognised to have a higher prevalence of left ventricular systolic impairment and heart failure symptoms at presentation (27, 33).

DISEASE PHENOTYPE

Pre-phenotypic Features

Predictive genetic testing as part of family screening has led to the identification of a growing population of genotype-positive phenotype-negative individuals. Although these individuals have not been shown to be at risk of disease-related complications such as life threatening malignant arrhythmias (23, 34, 35), they require ongoing clinical screening to detect phenotype development. Previous studies have described early "pre-phenotypic" features of sarcomere mutation carriers including ECG abnormalities (23, 24, 34), impaired LV relaxation (36), elongated mitral valve leaflets (37), or myocardial crypts (36–38) (**Figure 1**). Recent studies have identified independent predictors of developing a phenotype during follow up, including male sex or an abnormal ECG (23). However, our understanding of how these abnormalities relate to, and predict, the development of LV hypertrophy in childhood remains incomplete.

Phenotype at Diagnosis During Childhood

Left ventricular hypertrophy (LVH), as defined by a maximal left ventricular wall thickness >2 standard deviations (≥2 *Z*-score) above body surface area (BSA) corrected mean, is a pre-requisite

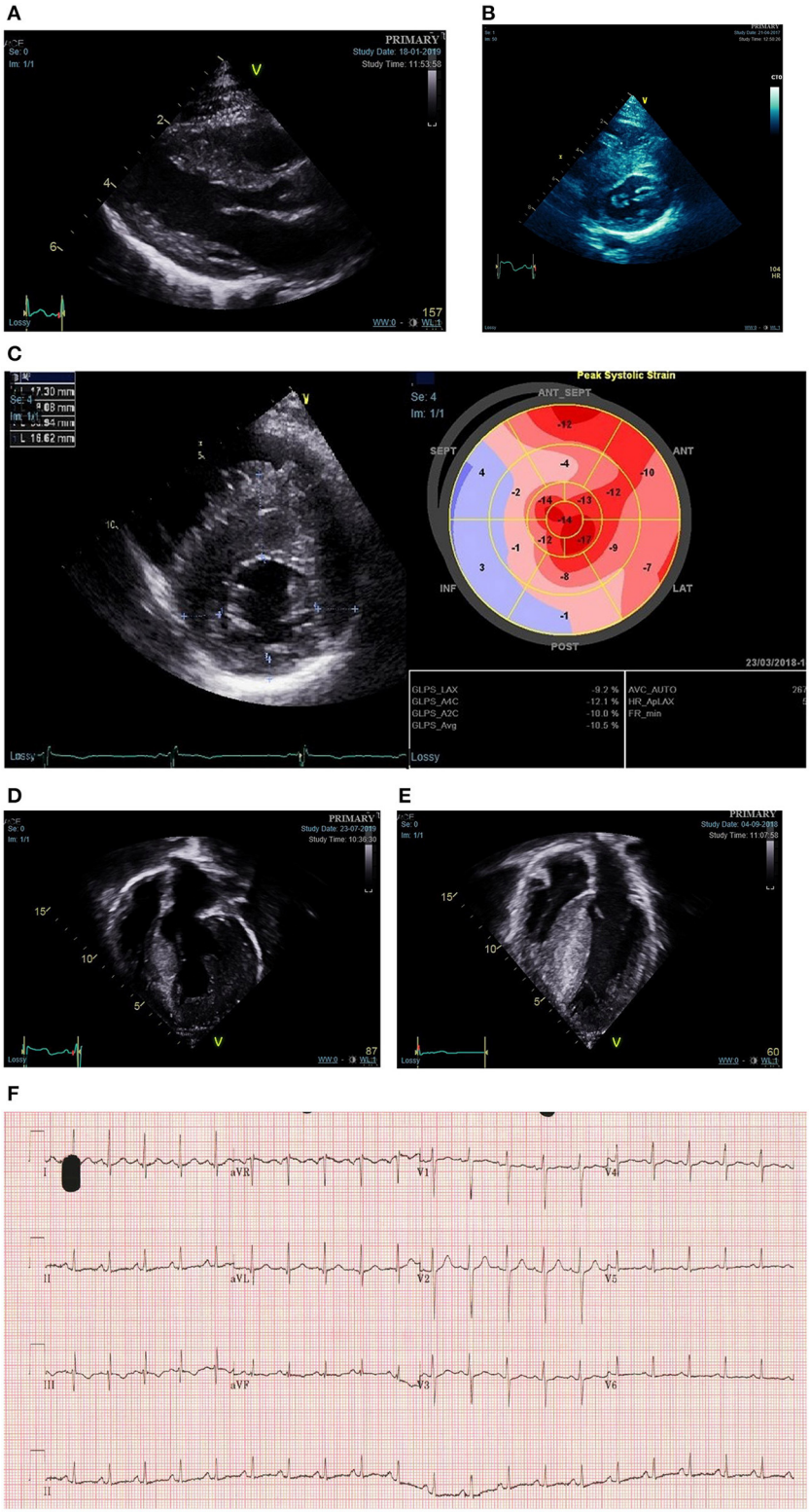

FIGURE 1 | Phenotypic spectrum of sarcomeric hypertrophic cardiomyopathy presenting during childhood. **(A)** Mild asymmetric LVH secondary to a disease causing MYH7 variant diagnosed in infancy through family screening. **(B)** Moderate-severe asymmetric LVH secondary to disease causing MYBPC3 variant diagnosed in infancy following referral with murmur. **(C)** Reduced longitudinal strain in a teenager with asymmetric LVH and familial disease. **(D)** Severe eccentric hypertrophy in a 10-year old with a disease causing alpha-tropomyosin variant. **(E)** Biventricular hypertrophy in a teenager with compound MYBPC3 variants. **(F)** ECG showing abnormal repolarisation (flat or negative T waves infero-laterally) in a child heterozygous for familial MYH7 variant with no LVH on echocardiogram.

for the diagnosis of childhood HCM (39, 40). The interpretation of all *z*-score thresholds is hampered by the use of different normative data for *z*-score calculations, each of which yields different *z*-scores for the same individual. It has, however, been suggested that the current BSA-corrected threshold for diagnosis in childhood is too low and should be increased to $Z \geq 6$ in line with the diagnostic threshold in adults of MLVWT ≥15 mm (or ≥13 mm for familial disease) (40). Sarcomeric HCM is typically characterised by asymmetric septal hypertrophy, although other morphologies of LVH (concentric, eccentric, or biventricular) more commonly associated with syndromic disease can also be seen in sarcomeric HCM (27–29).

The current paradigm, that the development of LVH in sarcomeric disease is uncommon during childhood, was based on a small study of 39 patients with familial disease in which increases in hypertrophy were seen more frequently in adolescence (41). However, studies have now shown that LVH can develop at any age in familial disease and indeed that the majority of childhood disease diagnosed through family screening met diagnostic criteria during pre-adolescence, with a significant proportion diagnosed in infancy (24, 31). One implication of the traditional paradigm is that younger children, if diagnosed, have phenotypically mild disease. More recent data from large, retrospective cohort studies have provided novel insights into the phenotype of childhood sarcomeric disease which challenge these assumptions. The HCM Risk-Kids and PRIMaCY cohorts included 1,072 and 572 patients, respectively, with non-syndromic childhood HCM, respectively, from international cardiac centres (42, 43). In both cohorts, the severity of hypertrophy was highly variable, and included a significant proportion with extreme hypertrophy (13% MLVWT ≥20 in HCM Risk-Kids). Importantly, the degree of hypertrophy was not dependent on age. Although diagnosis in infancy has traditionally been attributed to rare presentation of severe disease, recent studies have reported conflicting phenotypic findings in this population. In a UK cohort, MLVWT *Z*-score was similar for those presenting in infancy and later childhood (29), whilst recent data from the largely North American SHaRE registry reported a higher MLVWT *Z*-score for those presenting under the age of 1 (6). Together, these data suggest that the pattern and degree of hypertrophy in childhood sarcomeric disease is highly variable and can include both severe and mild LVH at any age (**Figure 1**).

Although LVH is a pre-requisite for diagnosis, additional morphologic abnormalities contribute to the overall phenotype of an individual patient. Left ventricular outflow tract (LVOT) obstruction [defined as a maximal LVOT gradient, as measured using Doppler echocardiography, above 30 mm (39)] is reported to be present in 22–60% (32, 44, 45) reflecting the heterogeneous nature of disease and differing definitions in published cohorts. LVOT obstruction (LVOTO) has been reported to be more common in those presenting in infancy (6). The mechanism for LVOTO is primarily systolic anterior motion of the mitral valve (SAM), with additional contributing factors in some cases including an anatomically narrowed LVOT, fixed LVOT obstruction and accessory mitral valve chords. Children with sarcomeric HCM are less likely to have complex LVOT obstruction due to a polyvalvulopathy with abnormal chordal attachments of the mitral valve as is seen in RASopathy syndromes (46). Global measures of LV systolic function (e.g., ejection fraction) are typically hyperdynamic; reduced ejection fraction is very rare in sarcomeric childhood disease and should prompt clinicians to search for underlying metabolic or syndromic disease (47, 48). However, myocardial deformation imaging may identify regional subclinical myocardial dysfunction, typically located in the area of maximal hypertrophy and longitudinal axis function is often impaired even in those with hyperdynamic systolic function (49, 50) (**Figure 1**). In contrast, diastolic dysfunction is commonly seen in established childhood disease and may even precede the development of hypertrophy (36). Data from the HCM-Risk Kids cohort shows that phenotypic features of severe disease (severe hypertrophy, LVOT obstruction, left atrial dilatation) often co-exist and that such patients are more likely to be symptomatic (42).

Disease Progression During Childhood

Childhood is a time of significant somatic growth and the cardiac phenotype of sarcomeric childhood HCM is highly variable and rapidly changing. However, our understanding of disease progression during childhood remains incomplete. Studies describing the follow up of childhood relatives (24, 31) or genotype-positive children (36, 51), have demonstrated that, in familial disease, increases in absolute and body-surface area corrected MLVWT occur throughout childhood. In a large single-centre cohort of over 1,000 paediatric first-degree relatives, screening appeared to identify two distinct populations (24). A small, but important, group of patients diagnosed before adolescent years with an accelerated progression of LVH, and a second, larger, group of patients diagnosed in later adolescence. In a minority of adult sarcomeric HCM patients (≈5%) evolution to a dilated hypokinetic phase with LV dilatation, systolic dysfunction and LV wall thinning has been described (52). Although this is the most common indication for heart transplantation in children with HCM (53), this is exceedingly rare in childhood-onset disease. However, several patients in the previously described familial screening cohort appeared to reach peak MLVWT during childhood, which could suggest that early onset disease may be associated with accelerated progression to end-stage disease (24). Future studies exploring the progression of sarcomeric childhood-onset disease are required.

Genotype-Phenotype Correlations

The presence of any disease-causing sarcomeric variant has been associated with earlier disease onset and more severe LVH (54, 55). However, efforts to explore genotype-phenotype correlations in sarcomeric HCM have been limited by significant genetic heterogeneity and variable or incomplete and age-related penetrance. **Table 1** describes reported genotype-phenotype correlations.

LONG TERM OUTCOMES AND MORTALITY

Early publications from small, highly selected tertiary centres reported that the long-term prognosis of childhood HCM was poor, with annual mortality rates of up to 7% (56, 57). Over time, larger, unselected population studies have reported lower mortality rates, which are more representative of the wider childhood HCM population. However, significant variability in outcomes exist and are largely dependent on age of presentation and underlying aetiology. European and North American population cohort studies have described children with "idiopathic" (presumed sarcomeric) disease to have a relatively good prognosis, with estimated 1 and 5-year survival of 94.4% (95% CI 92.5–96.4) and 82.2% (95% CI 76.2–88.2), respectively (28, 29). Infant-onset "idiopathic" disease was associated with worse prognosis (1-year mortality up to 14%) but for those that survived to 1 year after diagnosis, long-term outcomes were comparable to later-onset childhood disease (58). Heart-failure related deaths are responsible for the majority of mortality in infancy, whereas sudden cardiac death is the most common cause of death outside of infancy occurring at a rate of 1–2% per year (29, 42, 43, 58).

Whilst short term outcomes may be favourable, recent longitudinal data from the SHaRE registry have highlighted the cumulative morbidity and mortality associated with childhood-onset disease (6). Of children diagnosed between 1 and 18 years, 20% had experienced a cardiac event within 10 years of baseline assessment, which increased to 50% by 25 years of follow-up. Half of the early events were ventricular arrhythmias, whereas later events were more commonly heart failure-related or atrial fibrillation. Although end-stage disease is rarely observed during childhood, 40% had impaired LV systolic function by the age of 40 years and 20% had atrial fibrillation. This suggests that the lifelong burden of a childhood diagnosis is considerable and greater than previously appreciated. Compared to adults, children were more likely to experience malignant arrhythmias and twice as likely to require advanced heart failure treatments such as a cardiac transplantation or a left ventricular assist device. Interestingly, although infant-onset disease was associated with worse initial prognosis, for those that survived to 1 year, outcomes were better than childhood-onset disease with a lower cumulative incidence of heart failure, ventricular arrhythmias or atrial fibrillation providing further evidence that infant-onset disease is not necessarily a poor prognostic marker.

Predicting outcomes in childhood HCM is challenging because of the significant variability in age, aetiology, cardiac phenotype, and natural history. Multiple studies, in mixed childhood populations, have shown that presentation with symptoms of congestive cardiac failure is associated with higher cardiovascular mortality over follow up (28, 33, 59). Certain phenotypic features, largely associated with syndromic disease, including concentric LVH, biventricular hypertrophy, severe LVH and impaired systolic function, have also been associated with worse prognosis (27, 33, 58). Our understanding of the role that genotype plays in long-term outcomes is incomplete. The presence of a sarcomeric variant has been associated with a higher cumulative lifetime risk of experiencing an adverse cardiac event for both childhood and adult-onset HCM (54, 60, 61). However, the natural history of sarcomeric disease appears to differ by age of presentation with genotype positive children at increased risk of heart failure event whilst genotype positive adults are at increased risk of atrial fibrillation or all-cause mortality (6). A detailed discussion of the risk factors for arrhythmic events in childhood sarcomeric HCM can be found below but future studies exploring risk factors that predict mortality in childhood onset childhood HCM are required.

TABLE 2 | Proposed follow up and investigations for childhood Hypertrophic cardiomyopathy.

Time frame	Clinical review and investigations
6–12 months	Clinical review of symptoms, Transthoracic echocardiogram, 12 lead ECG Risk stratification of arrhythmic events
12–24 months	24-h ambulatory ECG—surveillance for malignant arrhythmias and to inform risk stratification
2–3 years	Cardiopulmonary exercise test (>7 years)—functional capacity assessment and arrhythmia provocation Cardiac Magnetic Resonance Imaging (CMRI) (>7 years)—LGE for fibrosis
At any time during follow up	Genetic testing
Additional investigations if indicated	Exercise stress echocardiography—investigation for latent LVOT obstruction in symptomatic patients

MANAGEMENT OF CHILDHOOD HCM

The management of children with sarcomeric HCM focuses on three main areas; family screening and counselling of family members, management of symptoms, and preventing disease-related complications. A proposed follow up schedule is shown in **Table 2**.

FAMILY SCREENING

Disease-causing variants in sarcomere protein genes are inherited in an autosomal dominant fashion and clinical or genetic screening is recommended for all first-degree relatives (39, 40). Although clinical screening was previously recommended to commence at the age of 10 or 12 years, in light of recent evidence from family screening cohorts described above, the 2020 AHA/ACC guidelines now endorse performing clinical screening at any age following the diagnosis of HCM in a first degree relative (40). Repeat clinical assessment is required throughout childhood into adulthood due to variable and age-related penetrance, with recent estimates suggesting that up to 50% of phenotype-negative mutation carriers may develop a phenotype over 15 years (23). If a disease-causing variant has been identified in the family, at-risk family members can be offered predictive genetic testing offering the possibility

of discharge from clinical follow up if they are found to be genotype negative.

SYMPTOM MANAGEMENT

Up to 70% of children with sarcomeric-HCM report cardiac symptoms, most commonly exertional or atypical chest pain, dyspnoea, palpitation or syncope (62). Chest pain is often multifactorial and can be caused by LVOT obstruction, increased wall stress due to elevated diastolic pressures leading to myocardial ischaemia, or microvascular abnormalities. Although systolic compression of epicardial and intramural vessels (myocardial bridging) is commonly seen, this is not usually of clinical importance (63). Heart failure symptoms are usually attributable to diastolic dysfunction with impaired filling. Syncope is commonly reported and has a variety of underlying mechanisms, including haemodynamic (vasovagal, LVOT obstruction or diastolic dysfunction) or arrhythmic. Investigation of the cause is important as unexplained syncope, presumed secondary to ventricular arrhythmias, is a risk factor for sudden cardiac death (42, 43, 64, 65). An understanding of the likely mechanism of symptoms is important to guide medical management.

Symptoms in the Presence of LVOT Obstruction

Management of LVOT obstruction is typically focused on relieving symptoms. Of note, echocardiographic findings of LVOT obstruction do not correlate well with symptom severity. Beta-blockers are considered to be the first line medical therapy and are well-tolerated (66). Alternative pharmacological therapies include disopyramide (67) and calcium channel blockers (68, 69), either alone or in combination. For those with refractory symptoms or fixed obstruction, surgical myectomy has been shown to be effective at both reducing the measured gradient and providing symptomatic relief during childhood with low operative morbidity or mortality in experienced centres (70, 71). A trend toward a higher incidence of re-operation in those undergoing myectomy during infancy has been reported (72). Other invasive gradient reduction therapies, such as alcohol septal ablation of radiofrequency ablation, remain experimental in childhood and are not recommended, as the long-term effects of such therapies are unknown (73). Treatment of asymptomatic LVOTO is controversial due to conflicting reports regarding its effect on long-term prognosis. Beta-blocker therapy may be initiated in young patients who are seemingly asymptomatic, reflecting difficulties in assessing symptomatology in this patient group.

Symptoms in the Absence of LVOT Obstruction

Symptoms in non-obstructive disease are usually secondary to impaired diastolic function or myocardial ischaemia caused by increased LV mass. Exercise echocardiography can be a useful investigation if symptoms are suggestive of LVOT obstruction to elicit latent obstructive disease (74), which has been reported in up to two thirds of children with sarcomeric disease. Treatment is largely empirical and includes beta blockers and calcium channel blockers.

SCD PREVENTION

HCM is characterised by a pro-arrhythmic triad of macroscopic and microscopic features including myocyte disarray, fibrosis and small vessel disease. Sudden cardiac death is the most common cause of death, outside of infancy, in non-syndromic childhood HCM and occurs more frequently than in adult cohorts (1–2 vs. 0.8%/year) (42, 43, 75). Identifying patients at highest risk of malignant arrhythmias is therefore a cornerstone of patient management. No medical therapy is currently recommended as preventative therapy for SCD in HCM. High dose beta-blockade (up to 6 mg/kg daily) has been described to reduce the risk of arrhythmic events in a small single centre study (66). However, these results have not been confirmed independently in paediatric or adult populations. Implantable cardioverter defibrillators (ICDs) have been shown to be effective at terminating malignant ventricular arrhythmias in children with HCM but at the expense of a higher rate of complications and inappropriate therapies compared to adult patients (65, 76, 77). As this younger group of patients will have ongoing exposure to these risks throughout their lifetime, and no device or programming strategies have been shown to reduce these risks (76), the ability to identify which patients are most likely to benefit from prophylactic ICD implantation is particularly important.

Risk Factors for SCD in Childhood HCM

Recent multi-centre collaborative studies [including HCM Risk-Kids (42), PRiMACY (43), and SHaRE registry (6)] have improved our understanding of the risk factors for SCD in childhood HCM. A previous meta-analysis of the published literature identified four major clinical risk factors for SCD in childhood HCM: previous ventricular fibrillation (VF) or sustained ventricular tachycardia (VT); unexplained syncope; NSVT; and extreme left ventricular hypertrophy (defined as a LV maximal wall thickness $\geq$30 mm or $Z \geq 6$) (78). Of note, although there is robust evidence to support the use of family history of SCD as a risk factor in adult patients, there was insufficient evidence to support its use in childhood. Possible explanations for this include a higher prevalence of *de novo* variants in childhood disease, low proportion of sarcomeric disease in the reported studies or insufficient reporting of family linkage. LVH is recognised as a major risk factor, but the most useful measure of hypertrophy remains unclear. Extreme hypertrophy was only associated with SCD in half of studies using this measure and recent studies in both adult and childhood populations have described a non-linear relationship between MWT and risk, meaning those with the most severe hypertrophy do not necessarily have the highest risk of events (43, 79). The meta-analysis also suggested additional risk factors, such as left atrial diameter and LVOT obstruction, may modify an individuals' risk similar to adult disease. **Table 3** describes the risk factors for SCD in childhood HCM described in the literature.

TABLE 3 | Risk factors for sudden cardiac death in childhood HCM.

Major risk factor	Clinical risk factor	Comment
Major risk factors	Previous VF/VT	Pooled HR 5.4 (95% CI 3.67–7.95, $P < 0.001$). Pooled OR 5.06 (95% 2.11–12.17, $P < 0.001$)
	Unexplained syncope	Pooled HR 1.89 (0.69–5.16, p 0.22). Pooled OR 2.64 (1.21–5.79, p 0.02)
	NSVT	Pooled HR 2.13 (95% CI 1.21–3.74, p 0.0009). Pooled OR 2.05 (96% CI 0.98–4.28, p 0.06).
	Extreme LVH	Pooled HR 1.8 (95% CI 0.75–4.32, p 0.19). Pooled OR 1.70 (95% CI 0.85–3.40, p 0.13). The most useful measure of LVH for risk stratification is unknown.
Other putative risk factors	LA dilatation	Left atrial size was not included as a major risk factor in the meta-analysis but a significant association has subsequently been reported in four studies (32, 42, 43, 80).
	LVOT gradient	The definition of LVOT obstruction varies in the literature. Increasing LVOT gradient has been linked to SCD (32, 45) and two large studies have described an inverse relationship between LVOT gradient and risk in childhood (42, 43).
	Family history of SCD	Only 1/10 studies reported a significant association between a family history of SCD and SCD event (81). Limited evidence to support its use as a risk factor during childhood.
	Age	The role of age in SCD is not fully understood. SCD risk has been reported to be increased in pre-adolescent years (9–14 yrs) (30) and children presenting in infancy are believed to be at lower risk (27, 58)
	12 lead ECG	Proposed 12 lead ECG features include; measures of LV hypertrophy (82) and abnormal repolarisation (83). An ECG risk score has been developed by Ostman-Smith et al. (83) but this was shown to have only moderate discriminatory ability in an external validation study (84).
	LGE on CMRI	LGE has been shown to increase during childhood and is associated with left ventricular hypertrophy (51). It is unclear if LGE is an independent risk factor for SCD (85, 86).
	Genotype	The role of genotype in SCD risk during childhood is not fully understood. In small cohorts, the presence of a pathogenic sarcomeric mutation has been described to be associated with worse prognosis (61) and certain genotypes associated with higher arrhythmic risk (87).

Adapted from Norrish et al. (78).

Risk Stratification for SCD Guidelines

Little controversy exists for patients who have previously experienced a malignant ventricular arrhythmia who are widely accepted to be at high risk of future arrhythmias and are recommended for a secondary prevention ICD device (39, 40). Current risk stratification guidelines recommend the use of four major risk factors extrapolated from adult studies to identify patients for primary prevention ICD implantation: extreme LVH; unexplained syncope; NSVT; and a family history of SCD. Previous studies have shown that the co-existence of multiple risk factors has a summative effect on risk (88) and guidelines recommend a threshold for ICD implantation [$\geq$1 risk factor in the AHA/ACC guideline (40) and $\geq$2 risk factors in the ESC guidelines (39)]. A validation of this approach in a cohort of childhood HCM patients from UK showed that, although the incidence of arrhythmic events increased with additional risk factors, it had a limited ability to distinguish between high and low risk patients leading to unnecessary ICD implantation in many (89).

Current practise for adult HCM patients has moved away from this approach to risk stratification, which provides relative risks for non-homogenous groups rather than individualised estimates of risk, and guidelines recommend the use of a validated risk prediction model (39, 40). HCM Risk-SCD uses readily available clinical risk factors to calculate individualised estimates of 5-year SCD risk to guide ICD implantation decisions (75). This model is not validated for use in paediatric populations but two separate paediatric risk models [HCM Risk-Kids (42) and PRiMACY (43)] have recently been published that offer clinicians the ability to calculate individualised estimates of 5-year risk of SCD using readily available clinical predictors for the first time (42, 43). The risk models differ in their age limit (16 years for HCM Risk-Kids vs. 18 years for PRiMACY), development cohort sample size (n = 1,072 HCM Risk-Kids vs. n = 572 PRiMACY) and approach to risk factor selection, which was either based on 30 years of published literature (HCM Risk-Kids) or association with the end-point on multivariable analysis in the model development cohort (PRiMACY). Nonetheless, the model predictor variables are identical (measures of LVH, left atrial diameter, NSVT, syncope), with the exception that PRiMACY also includes age as an independent predictor. These models have not yet been adopted by clinical guidelines but have been shown in independent external validation studies to have superior ability to identify patients at highest risk for arrhythmic events compared to current guidelines. The HCM-Risk-Kids model is available freely online (https://hcmriskkids.org) allowing clinicians to calculate individualised estimates of 5-year risk for their patients and perform independent external validation of the model. These models remain imperfect and future studies exploring the use of additional risk factors and serial clinical data to predict risk for childhood HCM are required.

DISEASE-MODIFYING TREATMENTS

To date, the management of HCM has focused on alleviating symptoms or preventing disease-related complications. However,

newly developed disease-specific therapies offer an exciting opportunity to modulate disease expression. Mavacamten, a novel myosin inhibitor, has been shown in Phase III clinical trials to reduce left ventricular outflow tract gradients and improve symptoms in adults with symptomatic obstructive HCM (90). It has also been shown to cause a dose-dependent reduction in serum markers of myocardial wall stress and injury (serum natriuretic peptide and cardiac troponin) in non-obstructive adult HCM patients (91) and prevent disease expression in mouse models (92). Although studies have not yet included childhood-onset disease, such medications offer the possibility of modulating disease expression both in patients with a phenotype and genotype-positive phenotype-negative individuals identified through screening.

CONCLUSION

Although the underlying aetiology of HCM presenting in childhood is heterogeneous, it is clear that it is primarily a disease of the cardiac sarcomere. Disease phenotype and progression is highly variable and includes mild and early phenotypes as well as severe disease presenting at a young age with accelerated progression to end-stage. Although overall prognosis during childhood is favourable, childhood onset disease is associated with considerable life-long morbidity and mortality, including a higher SCD rate during childhood than seen in adults. Management is currently focused on relieving symptoms and preventing disease-related complications, but the possibility of future disease-modifying therapies offers an exciting opportunity to modulate disease expression and outcomes in these young patients.

AUTHOR CONTRIBUTIONS

GN, EF, and JK contributed to conception and design of the review. GN and EF wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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REVIEW
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Cardiomyopathy in Genetic Aortic Diseases

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Genetic aortic diseases are a group of illnesses characterized by aortic aneurysms or dissection in the presence of an underlying genetic defect. They are part of the broader spectrum of heritable thoracic aortic disease, which also includes those cases of aortic aneurysm or dissection with a positive family history but in whom no genetic cause is identified. Aortic disease in these conditions is a major cause of mortality, justifying clinical and scientific emphasis on the aorta. Aortic valve disease and atrioventricular valve abnormalities are known as important additional manifestations that require careful follow-up and management. The archetype of genetic aortic disease is Marfan syndrome, caused by pathogenic variants in the *Fibrillin-1* gene. Given the presence of fibrillin-1 microfibers in the myocardium, myocardial dysfunction and associated arrhythmia are conceivable and have been shown to contribute to morbidity and mortality in patients with Marfan syndrome. In this review, we will discuss data on myocardial disease from human studies as well as insights obtained from the study of mouse models of Marfan syndrome. We will elaborate on the various phenotypic presentations in childhood and in adults and on the topic of arrhythmia. We will also briefly discuss the limited data available on other genetic forms of aortic disease.

Keywords: Marfan syndrome, HTAD, cardiomyopathy, arrhythmia, myocardial disease, *FBN1* gene

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INTRODUCTION AND DEFINITION OF THE DISEASES: MARFAN SYNDROME AND HERITABLE THORACIC AORTIC DISEASE

Heritable Thoracic Aortic Diseases (HTAD) encompasses a spectrum of genetic conditions in which aortic disease (aneurysms and dissections) has an underlying genetic trigger or familial occurrence. HTAD is classified as syndromic and non-syndromic. The genetic causes fall into several distinct groups of genes coding for (I.) components of the extracellular matrix (ECM) (*FBN1, COL3A1, LOX*); (II.) components involved in the TGFβ pathway (*TGFBR1 and 2, SMAD2 and 3 and TGFB2 and 3*); and (III.) components of the vascular smooth muscle cell apparatus (*ACTA2, MYLK, MYH11, PRKGA1*) (1). The main clinical entities with their respective genes and clinical features are listed in **Table 1**. Here, only those genes with a definitive or strong association with HTAD are listed. There are many more candidate genes on the horizon, and this list keeps growing.

The paradigm syndromic HTAD entity is Marfan syndrome (MFS). MFS is an inherited connective tissue disease caused by pathogenic variants in the Fibrillin-1 gene (*FBN1*), which codes for the ECM protein fibrillin-1. The condition was first described in 1895 by the French pediatrician Antoine Bernard Marfan who described a constellation of skeletal abnormalities characterized by

TABLE 1 | Main clinical features and genes* associated with Heritable Thoracic Aortic Aneurysm and Dissection.

	Disorder	Gene(s)	Main cardiovascular features	Additional clinical features
SYNDROMIC HTAD				
Extracellular matrix	Marfan Syndrome	*FBN1*	Aortic root aneurysm and dissection Mitral valve prolapse Ventricular dysfunction and arrythmia	Lens luxation Skeletal features
	Vascular Ehlers-Danlos syndrome	*COL3A1*	Aortic and major branching vessel dissection/rupture often without preceding dilatation	Thin, translucent skin Dystrophic scars Facial characteristics Bowel/uterine rupture Club feet Carotido-Cavernous fistulae
TGFβ-pathway	Loeys-Dietz syndrome	*TGFBR*1/2 *TGFβ*2/3 *SMAD3*	Aortic root aneurysm and dissection Arterial aneurysms and dissections Arterial tortuosity Mitral valve prolapse Congenital cardiac malformations	Bifid uvula/cleft palate Hypertelorism Craniosynostosis Pectus abnormalities Scoliosis Club feet Premature Osteoarthritis (*SMAD3*)
VSMC contractile apparatus	Smooth muscle cell dysplasia syndrome	*ACTA2 R189*	Patent ductus arteriosus Aorto-pulmonary window Aortic root dilatation	Congenital bilateral Mydriasis Moya-Moya like cerebral vessel anomalies Gut malrotation
NON-SYNDROMIC HTAD				
Extracellular matrix	FTAA	*FBN1* *LOX*	Aortic root aneurysm and dissection BAV (*LOX*)	Variable expression of some systemic features (pectus abnormalities, dural ectasia)
TGFβ-pathway	FTAA	*TGFBR*1/2 *SMAD2/*3 *TGF*β2/3	Thoracic aortic aneurysm and dissection Intracranial aneurysms Mitral valve prolapse	Variable expression of some systemic features
VSMC contractile apparatus	FTAA	*ACTA*2 *MYLK* *PRKG*1 *MYH11*	Cerebrovascular and coronary artery disease (*ACTA 2*) Patent Ductus Arteriosus (*MYH11, ACTA2*)	Livedo reticularis and iris flocculi (*ACTA2*) Gastro-intestinal abnormalities (*MYLK*)

BAV, bicuspid aortic valve; FTAA, familial thoracic aortic aneurysm; HTAD, heritable thoracic aortic aneurysm and dissection; VSMC, vascular smooth muscle cell.
**Only genes with a strong or definitive association are listed.*

joint contractures and conspicuously long fingers (arachnodactyly) in a young girl (2). It took over 50 years for the clinical picture of Marfan syndrome to be more clearly defined in the seminal work of Victor McKusick. He described the condition as a connective tissue disease with cardiovascular involvement. Without knowing the underlying molecular defect, he very accurately reported that "Clinically, Marfan syndrome behaves as an abiotrophy of some connective tissue" (3). By this time, cardiovascular involvement had been consistently reported, along with the skeletal and ocular organ systems' involvement. The concept of an abnormality in elastic fibers in the aorta as a cause for the characteristic aortic aneurysms and dissections was put forward. In addition, involvement of the veins, the heart valves and also the endocardium and myocardium were suspected. The latter fact is of particular value in the context of this review.

Unraveling the structural components of connective tissue again took several decades. Evidence for the link between connective tissue and the clinical entity of Marfan syndrome was first provided by immunohistochemic studies using antibodies for fibrillins, showing deficiencies in the amount and distribution of microfibrillar fibers in skin samples from patients with MFS (4). The identification of pathogenic missense variants in the *FBN1* gene in two patients with Marfan syndrome in 1991 provided final confirmation (5). Fibrillins are large structural macromolecules that contribute to the integrity and function of all connective tissues. According to the initial concept, fibrillin microfibrils mainly served as a scaffold for elastic fiber formation. Biochemical investigations and genetic evidence from both humans and mice have now uncovered many more functions of fibrillin microfibrils. Today, we know that fibrillin microfibrils have essential tissue-specific architectural functions beyond serving as scaffolds for elastin deposition. More recently, an important functional role of fibrillin microfibrils has emerged: fibrillin microfibrils target and sequester members of the TGFβ superfamily of growth factors. In this manner, the structures of fibrillin microfibrils collaborate with biological functions to shape and maintain connective tissues (6). The combined structural and functional role of fibrillins nicely illustrates the current concept of mechanobiology underlying the pathophysiology of cardiovascular disease in MFS. Through interactions between vascular smooth muscle cells (in the aorta) or cardiomyocytes (in the myocardium) and the ECM, the cells can sense changes in mechanical forces of the ECM. These mechanical signals are converted into biochemical or electrical signals, thereby enabling a responsive cellular adaption and remodeling. This process, which is bidirectional, is called mechanobiology. The composition of the ECM and

the proportion and the expression of each protein can have a profound influence on cardiac structure and compliance that will determine its hemodynamic functions. One of the major myocardial ECM components is collagen, which will, when present in excessive concentrations lead to myocardial fibrosis and distortion of the myocardial architecture. Fibrosis is prevalent in many acquired cardiac diseases and underlies several adverse cardiac events, such as heart failure, arrhythmia, and death. Increased fibrillin-1 expression has been reported in the context of myocardial fibrosis (7) and gene expression studies have targeted genes involved in the ECM as highly enriched in patients with cardiomyopathy (CMP) with fibrosis and cardiac remodeling (8). Monogenic forms of CMP caused by pathogenic variants in genes encoding for ECM structural components are scarce. Cases of non-compaction CMP caused by *FBN1* pathogenic variants have been reported (9). Further research in this field is highly relevant, not only to identify potential other genes involved but also since several proteins represent candidate therapeutic targets to prevent or reverse fibrosis (10).

A detailed description of the role of the ECM in the myocardium can be found in a recent review by Frangogiannis (11).

The diagnosis of MFS is based on the identification of clinical manifestations, as defined in the revised Ghent nosology (12). The extent, severity and age of onset of clinical manifestations are highly variable, ranging from severe cardiovascular involvement at birth in the neonatal form to patients developing manifestations only in mid-life. The estimated prevalence of Marfan syndrome is 1 in 3.000–5.000 individuals, with no ethnic or sex predilection (13). Prognosis is mainly determined by progressive dilation of the aorta, leading to aortic dissection and death at a young age. Mean survival of untreated patients is about 40 years. Fortunately, improved management and ongoing research have led to a significant increase in life expectancy of at least 30 years (14, 15) which does not imply that life expectancy in MFS is normal. A recent population study demonstrated a median age at death in MFS patients of 50 years, which is 8–13 years lower than in the general population (16). A critical factor in improving prognosis is the early identification of patients with Marfan syndrome. Precipitating factors reported to accelerate progressive dilatation or dissection include elevated blood pressure, intense physical exercise and pregnancy (17, 18).

CARDIOMYOPATHY AND ARRHYTHMIA IN MARFAN SYNDROME

When referring to Marfan syndrome CMP, two different clinical entities should be distinguished: (1) Heart failure in very young children with MFS (neonates and infants) (2) CMP in classical MFS. We will discuss both entities separately in the next sections.

Cardiomyopathy in Neonatal and Infantile Marfan Syndrome

Neonatal MFS (nMFS) is a term usually reserved for very early clinical presentations of MFS even though some patients may present after the 1st month of life (19). The exact prevalence of nMFS is unknown but is much lower than the prevalence of classical Marfan syndrome. The majority of these patients (90–95%) carry a de novo variant in the so-called "neonatal region" (exons 24–32) with a cluster of variants in exons 25–26 (20). Children with nMFS have a typical appearance with dolichocephaly, progeroid appearance, arachnodactyly, crumpled ears, joint contractures and pectus abnormalities (**Figure 1A**). Some children will exhibit congenital lung emphysema and ocular abnormalities. Unlike classical MFS, the most prominent cardiac problem in children with nMFS is tricuspid and mitral valve prolapse, usually with severe progressive regurgitation leading to congestive heart failure (**Figures 1B–E**) (20–22). Aortic root dilatation is also commonly present in children with nMFS but does not account for the most significant morbidity and mortality in this age group. Most children with nMFS die within the 1st year of life of cardiac failure (20), although the number of survivors into teenage years is increasing thanks to improvement in care (23, 24).

Children with the infantile form of MFS usually present a less severe course (25). These children might be diagnosed at a very young age (even during the first months of life), and the phenotype is similar to that of nMFS. However, from a cardiovascular perspective, the infantile form of MFS resembles a more severe subform of classical Marfan syndrome. They show severe aortic root dilation at a young age, but atrioventricular valve dysfunction is typically less prominent than in the neonatal form (21, 26). These children usually present left ventricular dilatation, even with mild valvular regurgitation, but preserved left ventricular function.

Cardiomyopathy in Classic Marfan Syndrome

For obvious reasons, cardiovascular research and management recommendations for classic MFS have heavily focused on aortic disease. Interest in studying myocardial involvement was raised in the nineties with a study from Savolainen and colleagues, indicating abnormal diastolic function in children with MFS, assessed by cardiac magnetic resonance (CMR) (27).

Moreover, several (historical) series on survival in MFS have listed heart failure as one of the leading causes of death. Estimates vary between 5 and 30% (16, 28) putting heart failure at least at an equal level as aortic dissection. End-stage heart failure necessitating heart transplantation in patients with MFS has been reported in several case reports and small series (29–31). In most of these patients, heart failure was triggered by underlying severe valvular disease (aortic and/or mitral valve regurgitation).

In addition to these extrinsic (secondary) causes of heart failure, several reports from independent researchers have shown intrinsic myocardial dysfunction in MFS. The reported prevalence of what is now known as Marfan CMP ranges from 3% (32) to 68% (33) across different series, depending on the definition and population characteristics. Involvement of both left and right ventricles with systolic and diastolic dysfunction has been reported (27, 34–39) (**Table 2**). Although myocardial dysfunction is mostly mild and subclinical and does not progress

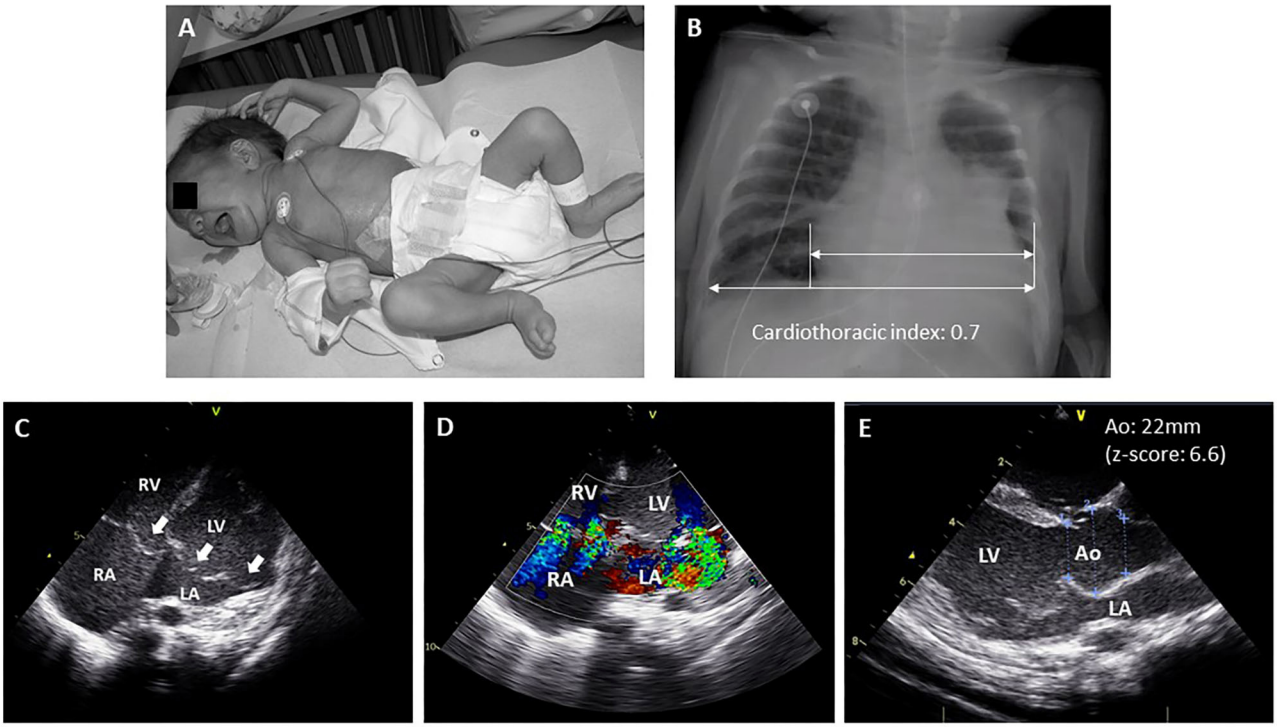

FIGURE 1 | Typical manifestations of neonatal Marfan syndrome. **(A)** Child with neonatal MFS showing arachnodactyly, long feet, crumpled ears, lipodystrophy and mild pectus excavatum. **(B)** Chest X-ray showing cardiomegaly and mild scoliosis. **(C)** Echocardiographic four-chamber view showing mitral and tricuspid valve prolapse (white arrows). **(D)** Echocardiographic apical four-chamber color doppler view showing moderate-severe mitral and tricuspid valve regurgitation. **(E)** Echocardiographic parasternal long axis view showing enlargement of the sinus of Valsalva. Ao, aorta; LA, left atrium; LV, left ventricle; RV, right ventricle; RA, right atrium.

much over time, some patients will present in overt heart failure. A possible link between intrinsic CMP and an unfavorable course in the event of an additional hemodynamic trigger such as valvular dysfunction and/or aortic root replacement has not yet been demonstrated but seems plausible. Whether these hemodynamic changes trigger myocardial fibrosis, as seen in other types of CMP (50), is also not clear in MFS. A small study in children with MFS and Loeys-Dietz syndrome (LDS) showed increased left- and right ventricular volumes and diffuse myocardial fibrosis on CMR in comparison to healthy control subjects (51). Further evidence is necessary to elucidate whether diffuse fibrosis is present in MFS and influences clinical outcome. Whether the type of underlying pathogenic *FBN1* variant plays a role in defining the risk for developing CMP is still unclear. Two independent studies observed a higher incidence of left ventricular dilation and decreased left ventricular function in patients carrying non-missense variants (46, 49), which is in line with recent data on genotype-phenotype correlations in patients with Marfan syndrome, indicating a worse cardiovascular phenotype in patients harboring non-missense variants predicted to result in a haploinsufficient effect (52).

Arrhythmia in Marfan Syndrome

Next to impaired function, arrhythmia may be considered as another manifestation of myocardial disease in MFS (**Figure 2**). Most of the evidence for arrhythmia in MFS comes from the study of adult cohorts, in which children were only occasionally included (**Table 3**). In these studies, significant ventricular ectopy (defined as >10 premature ventricular contractions per hour) was found in 20–35% of the patients (33, 49, 56). A slightly lower percentage of patients with MFS (10–25%) also present non-sustained ventricular tachycardia (NSVT) on 24h ambulatory monitoring (33, 49, 54, 56). Ventricular tachycardia (VT) and sudden cardiac death (SCD) have respectively been reported in 7–9 and 4% of the patients (33, 55, 56, 58).

One of the first studies of arrhythmia in children with MFS took place in the early 80s (53). In this study, eight of the 24 children with MFS (33.3%) presented with ventricular arrhythmia, three of whom showed ventricular tachycardia. Ventricular arrhythmia was associated with mitral valve prolapse and prolonged repolarization time. Another interesting early observation comes from the first (and only) randomized trial assessing the effect of propranolol on aortic root dilatation in patients with Marfan syndrome (59). Two deaths were observed in the control group of this trial, one 14 year old boy and one 18 year old women, both of which had mitral-valve prolapse and a history of paroxysmal tachyarrhythmia. Aortic dissection was excluded in both postmortem. While this study lacks power to draw meaningful conclusions from this observation, the history of arrhythmia is remarkable and one may even hypothesize that

TABLE 2 | Overview of the studies assessing cardiac function in Marfan syndrome.

Author Year	Subjects (mean age/range)	Method	Main findings
CARDIOMYOPATHY			
Savolainen (27) 1994	22 MFS (3-14.5 yr) 22 control	Echocardiography Cardiac MRI	Similar LV diameter and systolic function LV relaxation impairment in MFS
Porciani (40) 2002	20 MFS (29.5 yr) 8 MASS 28 controls	Echocardiography	Similar LV diameter and systolic function LV diastolic dysfunction in MFS
Chatrath (41) 2003	36 MFS without valvular disease	Echocardiography	19% LV dilatation Normal systolic function
Meijboom (34) 2005	234 MFS (29 yr)	Echocardiography	Normal systolic function and ventricular dimensions in most of the patients. Mild involvement in a subgroup
De Backer (36) 2006	26 MFS (32 yr) 26 controls	Echocardiography Cardiac MRI	Mild but significant impairment of LV systolic and diastolic dysfunction in MFS
Das (35) 2006	40 MFS (17 yr) 40 controls	Echocardiography	Impaired relaxation independent of aortic root dilation
Rybczynski (37) 2007	55 MFS 86 controls	Echocardiography	Reduced systolic and early diastolic tissue doppler velocities in adults with MFS
Kiotsekoglou (38) 2008	66 MFS (15-58 yr) 61 controls	Echocardiography	LV systolic dysfunction is significantly reduced in MFS
Kiotsekoglou (42) 2008	72 MFS (32 yr) 73 controls	Echocardiography	Significant biventricular diastolic and biatrial systolic and diastolic dysfunction in MFS patients
Kiotsekoglou (43) 2009	66 MFS (15-58 yr) 61 controls	Echocardiography	Primary impairment of RV systolic function in MFS
Alpendurada (39) 2010	68 MFS (33.9 yr)	Cardiac MRI	Primary cardiomyopathy in a subgroup of MFS patients
de Witte (44) 2011	144 MFS 19 controls	Cardiac MRI	Lower RV- and LVEF 9% LVEF<45% Result independent of aortic elasticity of β-blocker use
Scherpetong (45) 2011	50 MFS (35.2 yr) 50 controls	Echocardiography Longitudinal, FU: 4 yr	Lower RV and LV strain rate in MFS No progression during FU in the majority.
Aalberts (46) 2014	183 MFS (33.5 yr)	Echocardiography	LV dilatation is more frequent in patients with a non-missense *FBN1* pathogenic variant
Campens (47) 2015	19 MFS (adults)	Echocardiography Longitudinal, FU: 6 yr	No further echocardiographic deterioration of LV function during FU
Gehle (48) 2016	217 MFS (30 yr)	Echocardiography	Increased Nt-ProBNP levels Increased LV diameters LV diastolic dysfunction
Muiño-Mosquera (49) 2020	86 MFS (36.3 yr) 40 controls	Echocardiography Nt-ProBNP	Increased Nt-ProBNP levels, increased LV diameters and decreased RV function Patients after aortic surgery of with valvular disease more affected

FU, Follow-up; LV, Left ventricle; LVEF, Left ventricle ejection fraction; MASS, mitral valve, aortic, skin, skeletal features; MFS, Marfan syndrome; MRI, Magnetic Resonance Imaging; Nt-ProBNP, N-terminal pro-hormone brain natriuretic peptide; RV, Right ventricle; yr, years.

propranolol had a protective effect in the treatment group. A more recent study, an ancillary analysis of the Pediatric Heart Network (PHN) Marfan trial (57) also studied arrhythmia in children with MFS. The primary aim of the PHN Marfan trial was to compare aortic outcome in 608 children with MFS, randomly assigned to treatment with atenolol or losartan. As part of this study, a subgroup of patients ($n = 274$) underwent 24h ambulatory monitoring. Ventricular ectopy was present in 7% of these children, but (NS)VT was not observed. The prevalence of ventricular arrhythmia in these two studies is clearly different but probably reflects clinical variability among the cohorts. On the contrary, these studies show that ventricular arrhythmia, although uncommon, can present at an early age. Physicians taking care of children with MFS should also be vigilant for these complications.

The mechanisms underlying severe ventricular arrhythmia in MFS are not clear yet. Judging from the numbers above, it seems that age might play an important factor, although this has not been clearly shown (49). So far, an enlarged LV diameter appears to be the most consistent independent factor associated with an arrhythmic event (33, 49, 55). Other factors like mitral valve prolapse, mitral valve regurgitation and previous aortic surgery have also been associated with ventricular arrhythmia in a variable amount of studies (33, 49, 56, 60). Two studies from the Hamburg Marfan center indicated that NT-proBNP level is the strongest independent predictor of arrhythmogenic events

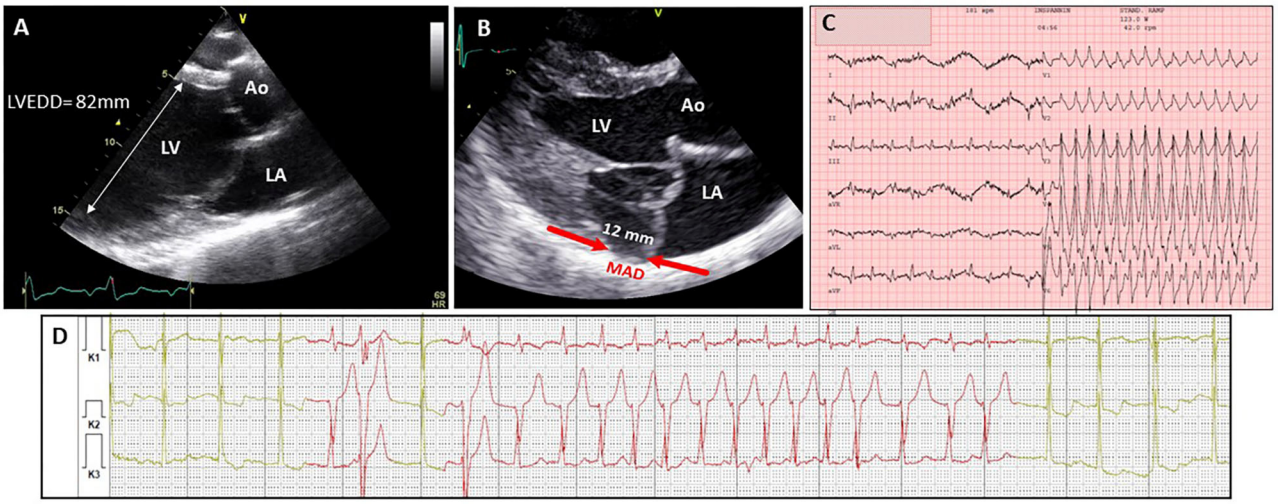

FIGURE 2 | Different manifestations of myocardial disease in classical Marfan syndrome. **(A)** Echocardiographic image (2D TTE PSLAX view) in a 16 yr old male showing severe dilatation of the left ventricle. **(B)** Echocardiographic image (2D TTE PSLAX view) showing mitral annular disjunction. **(C)** ECG recordings in 28 yr old male showing ventricular tachycardia followed by presyncope during exercise test 6 m after mitral valve surgery and aortic root replacement **(D)** ambulatory electrocardiogram recording in a 57 yr old female with frequent episodes of non-sustained ventricular tachycardia. Ao, aorta; LA, left atrium; LV, left ventricle; LVEDD, left ventricular end-diastolic diameter; MAD, mitral annular disjunction.

TABLE 3 | Overview of the published papers evaluating ventricular arrhythmia in MFS.

Author Year	Subjects (mean age/range)	Method	Main findings
VENTRICULAR ARRHYTHMIA			
Chen (53) 1985	24 MFS (children)	Echocardiography AECG	Serious ventricular dysrhythmia can occur in children with MFS with or without valve disease. The dysrhythmia appears to progress with age
Savolainen (54) 1997	45 MFS (34 yr) 45 controls	AECG	Patients with MFS have a higher prevalence of cardiac dysrhythmias than healthy persons
Yetman (33) 2003	70 MFS (0–52 yr)	Echocardiography ECG AECG FU: 24 yr	Sudden death occurring in 4% of MFS patients LV dilation may predispose to alterations of repolarization and fatal ventricular arrhythmias
Hoffman (55) 2013	77 MFS (36.1 yr)	Echocardiography ECG AECG NT-ProBNP	NT-ProBNP predicts adverse arrhythmogenic events in patients with MFS
Aydin (56) 2013	80 MFS (42 yr)	Echocardiography ECG AECG NT-ProBNP	MFS is associated with an increased risk for arrhythmia. Risk factors: Ventricular arrhythmia on ECG, signs of myocardial dysfunction and pathogenic variants in exons 24–32
Mah (57) 2018	274 MFS (10.8 yr)	Echocardiography AECG	VE and supraventricular ectopy is rare in children with MFS. Increased LV diameter is related to ventricular ectopy
Muiño-Mosquera (49) 2020	86 MFS (36.3 yr) 40 controls	Echocardiography ECG AECG Nt-ProBNP	VE and NSVT were more frequent in MFS than in age- and sex-matched controls NSVT was independently associated with increased LV diameter and VES.

AECG, ambulatory electrocardiogram; ECG, electrocardiogram; FU, Follow-up; LV, Left ventricle; MFS, Marfan syndrome; NSVT, non-sustained ventricular tachycardia; VE, ventricular ectopy; yr, years.

(55, 56). In our own study, NT-pro-BNP levels were also elevated in patients presenting NSVT, although not significantly (49).

Lately, mitral annular disjunction (MAD), defined as the separation between the posterior mitral valve leaflet hinge point and the left ventricular myocardium, has gained interest in patients with MFS. A recent study shows that 46% of patients have MAD and that presence of MAD is associated with a worse aortic and mitral outcome (61). In non-MFS subjects, MAD

has also received particular attention as a potential marker or substrate of ventricular arrhythmia and SCD (62). In our recent study in 142 patients with MFS, MAD was present in 36% of the cohort and was associated with the presence of VT and SCD (manuscript in press, JAMA Cardiology). Ventricular ectopy in patients with mitral valve prolapse and MAD is presumed to be partially due to regional stretch leading to fibrosis of the papillary muscles (63). Whether the same underlying mechanism is present in MFS is not clear yet and deserves further study.

Besides an abnormal substrate, triggering factors might also play a role in developing arrhythmia in MFS. Subtle ECG changes have been identified in patients with MFS independent of aortic root diameter, mitral and/or tricuspid valve prolapse or chamber dimension and function. Patients with MFS display slightly prolonged PQ- and QTc-intervals compared to healthy controls (49, 54).

Atrial arrhythmia in MFS has been given less attention. Atrial fibrillation seems to be more common in MFS than in the general population and seems to occur at a younger age (49, 64). Other types of atrial arrhythmias have been described in MFS, mainly re-entry tachycardia, but it does not seem more frequent in MFS than in the general population (57).

THE MYOCARDIUM IN (MARFAN) MOUSE MODELS

The presence of fibrillin-1 in the myocardium has clearly been evidenced in wild-type mice. Histology shows more abundant amounts in the atria compared to the ventricles and a distinct spatial arrangement in the ventricular myocardium with more fibrillin-1 in the inner trabeculated part when compared to the outer trabeculated part (65). The inner myocardium is more prone to shearing forces during ventricular contraction, and connective tissue aligning these lamellae play a role in providing mechanical coupling and preventing overextension (66). Based on the known elastic properties of fibrillin-1 and its observed spatial arrangement at the level of the inner myocardium, it is conceivable that fibrillin-1 provides the required elasticity to the myocardial tissue allowing shearing of the muscle lamellae. This hypothesis is supported by the limited presence of elastic fibers in the myocardium, making fibrillin-1 fibers the most important myocardial ECM component with elastic properties (67). In addition, the role of fibrillin-1 in providing elasticity to the myocardium is also observed in the *fbn1*$^{mgR/mgR}$ Marfan mouse model. A decrease in passive filling properties of the left ventricle in this model suggests an impaired elastic recoil of the left ventricle (68). It is assumed that the underlying abnormality in the *FBN1* gene in MFS results in an impaired signaling function of fibrillin microfibrils in the ECM and that mechanical factors such as volume- or pressure overload are not correctly compensated which in turn leads to myocardial dysfunction. An example of pressure overload is provided by Rouf and colleagues who observed CMP after partial ligation of the aortic arch in *fbn1*$^{C1039G/+}$ mice (69). Volume overload caused by valvular regurgitation (both mitral and aortic valve) results in dilated CMP in the same mouse model (70).

These observations support the concept of mechanobiology as a possible cause for CMP (71). As already mentioned, the model of abnormal mechanobiology has also been introduced in recent years to explain aortic pathology in MFS (72).

In addition to the evidence for functional impairment of the myocardium in mouse models for MFS, some interesting morphological alterations are also worth mentioning. In the *fbn1*$^{mgR/mgR}$ mice, an age-dependent decrease in myocardial compaction was noted on routine staining compared to WT mice sections (65). As mentioned above, fibrillin-1 fibers align the periphery of inner myocardial muscle lamellae in WT tissue. It appears that fibrillin-1 functions as a glue in the trabeculated myocardium, strengthening intercellular connections through cell-ECM-cell interactions by forming molecular bridges between the pericellular and interstitial ECM. Abnormal fibrillin-1 fibers may lead to loosened connections or non-compaction. Interestingly, left ventricular non-compaction has also been linked to pathogenic *FBN1* variants in humans (9, 73). Next to the loss of myocardial compaction, macroscopic inspection of the right ventricle free wall in the *fbn1*$^{mgR/mgR}$ mouse model revealed the presence of (multiple) pseudoaneurysms. In WT mice, fibrillin-1 fibers cross the entire right ventricle free wall from lumen to pericardium. In the setting of MFS, reduced amounts of fibrillin-1 fibers may result in the formation of a gap crossing the entire right ventricle free wall. To our knowledge, there is no confirmation of such findings in human Marfan disease, but this definitely deserves further research.

RECOMMENDATION FOR FOLLOW-UP AND TREATMENT

Myocardial disease is an upcoming problem in MFS, especially in adults. As indicated above, a subgroup of children is also at risk. Careful monitoring of myocardial function and potential complications such as arrhythmia in patients with MFS is warranted. Yearly echocardiographic evaluation and follow-up should include assessment of biventricular systolic and diastolic function. Whether CMR will aid in risk stratification in patients with MFS still needs to be elucidated. Patients with enlarged left ventricular diameter, patients with MAD and patients with palpitations, (pre)syncope, or chest pain can benefit from an ambulatory electrocardiogram. As in general recommendations NT-pro-BNP levels are useful to monitor heart failure and may be useful for risk stratification for arrhythmia in Marfan syndrome (49, 55, 56).

There is currently no evidence that treatment of arrhythmia and heart failure in patients with MFS should differ from the guidelines for other non-MFS patients. If congestive heart failure is present as a result of valvular dysfunction, afterload-reducing agents can improve cardiovascular function. Whether losartan (or other angiotensin receptor blockers), drugs known for their beneficial effect in heart failure should be considered a first line choice in these patients is not known. In a small study with losartan in patients with MFS, we did not observe a significant effect on LV dimensions- and function (74). Indications for

surgical intervention for valvular disease should follow the general guidelines (75).

End-stage heart failure is uncommon in patients with MFS but heart transplantation may be considered. Although early results with this procedure in MFS were not good (30), subsequent reports were more encouraging (29). A major concern relates to complications occurring in the distal aorta, which are mostly (but not exclusively) occurring in patients with pre-existing aortic complications (31, 76). Given the inherent fragility of the aortic tissue, the use of assist devices should be limited, although successful cases have been reported (77).

The role of β-blocker therapy for the treatment of arrhythmia or prevention of SCD in MFS is not clear yet. In one study, two of the three patients presenting SCD were under treatment with β-blocker (33). In our study, one patient presenting VT showed progressive ventricular ectopy under β-blocker treatment and episodes of sustained VT were only controlled after treatment with amiodarone (49). In the absence of specific risk factors to stratify patients at risk of VT or SCD, indications for implantable defibrillator should follow the general guidelines (78).

EVIDENCE OF CARDIOMYOPATHY AND ARRHYTHMIA IN OTHER HTAD

There is very scarce literature on myocardial disease and arrhythmia in HTADs and evidence of myocardial dysfunction in this group of diseases, mainly comes from case reports and casual description from registries.

LDS was first described in 2005 as a connective tissue disorder with important vascular involvement (79). Shortly thereafter, a case report describing a patient carrying a pathogenic variant in *TGFBR1* with heart failure necessitating heart transplant was published (80). Several other case reports mentioned both heart failure and SCD in patients carrying variants in other genes: *TGFB2, TGFBR2* and *SMAD3* (81–84). Myocardial disease with left ventricular hypertrophy in 16% and VE in 19% of the patients carrying variants in *SMAD3* was already reported in one of the first case series characterizing patients with pathogenic variants this gene (85). Only one systematic study evaluating repolarization abnormalities in patients carrying variants in *TGFBR2* has been published (86). In this study, two patients presented SCD and 47% of patients presented abnormal repolarization characterized by slight prolongation of the QTc interval, abnormal ST-segment and abnormal T-U wave.

CONCLUSION

Findings based on human studies and from mouse models provide increasing evidence for the clinical relevance of CMP in genetic aortic disease, but more data are required for further confirmation and delineation.

Careful monitoring of myocardial function and potential consequences such as arrhythmia in patients with MFS and other HTAD is warranted. Further study to understand the underlying pathophysiology of myocardial disease is necessary to identify better treatment targets and improve patient's outcome.

AUTHOR CONTRIBUTIONS

JDB and LM-M wrote and reviewed this paper. Both authors contributed to the article and approved the submitted version.

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REVIEW
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Overview of Cardiomyopathies in Childhood

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Paediatric cardiomyopathies are a heterogenous group of rare disorders, characterised by mechanical and electrical abnormalities of the heart muscle. The overall annual incidence of childhood cardiomyopathies is estimated at about 1 per 100,000 children and is significantly higher during the first 2 years of life. Dilated cardiomyopathies account for approximately half of the cases. Hypertrophic cardiomyopathies form the second largest group, followed by the less common left ventricular non-compaction and restrictive phenotypes. Infectious, metabolic, genetic, and syndromic conditions account for the majority of cases. Congestive heart failure is the typical manifestation in children with dilated cardiomyopathy, whereas presenting symptoms are more variable in other phenotypes. The natural history is largely influenced by the type of cardiomyopathy and its underlying aetiology. Results from a national population-based study revealed 10-year transplant-free survival rates of 80, 62, and 48% for hypertrophic, dilated and left ventricular non-compaction cardiomyopathies, respectively. Long-term survival rates of children with a restrictive phenotype have largely been obscured by early listing for heart transplantation. In general, the majority of adverse events, including death and heart transplantation, occur during the first 2 years after the initial presentation. This review provides an overview of childhood cardiomyopathies with a focus on epidemiology, natural history, and outcomes.

Keywords: cardiomyopathy, paediatric, epidemiology, long-term outcomes, risk factors, sudden cardiac death, heart transplantation

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INTRODUCTION

Paediatric cardiomyopathies form an uncommon and heterogenous group of disorders, which are characterised by structural, mechanical, and electrical abnormalities of the heart muscle (1, 2). Aetiologies are diverse, and include infections, toxin exposure, tachyarrhythmias, genetic mutations, and underlying metabolic or neuromuscular disorders (1–6). Large population registries together with national and multicentre studies have contributed considerably to the increasing knowledge on epidemiology and outcomes of childhood cardiomyopathies (5, 7–24). The overall annual incidence is estimated at about 1 per 100,000 children, with a significantly higher incidence during the first 2 years of life (7, 17, 18). Dilated and hypertrophic cardiomyopathies are the most common types, whereas left ventricular non-compaction and restrictive cardiomyopathy occur less frequently (7, 17). Arrhythmogenic ventricular cardiomyopathy is rarely diagnosed during childhood and will not be discussed in this article. The terms dilated, hypertrophic, restrictive, and non-compaction depict different phenotypes (see **Figure 1**) and thereby assist in grouping cardiomyopathies, however they do not describe specific disease entities. The European Society

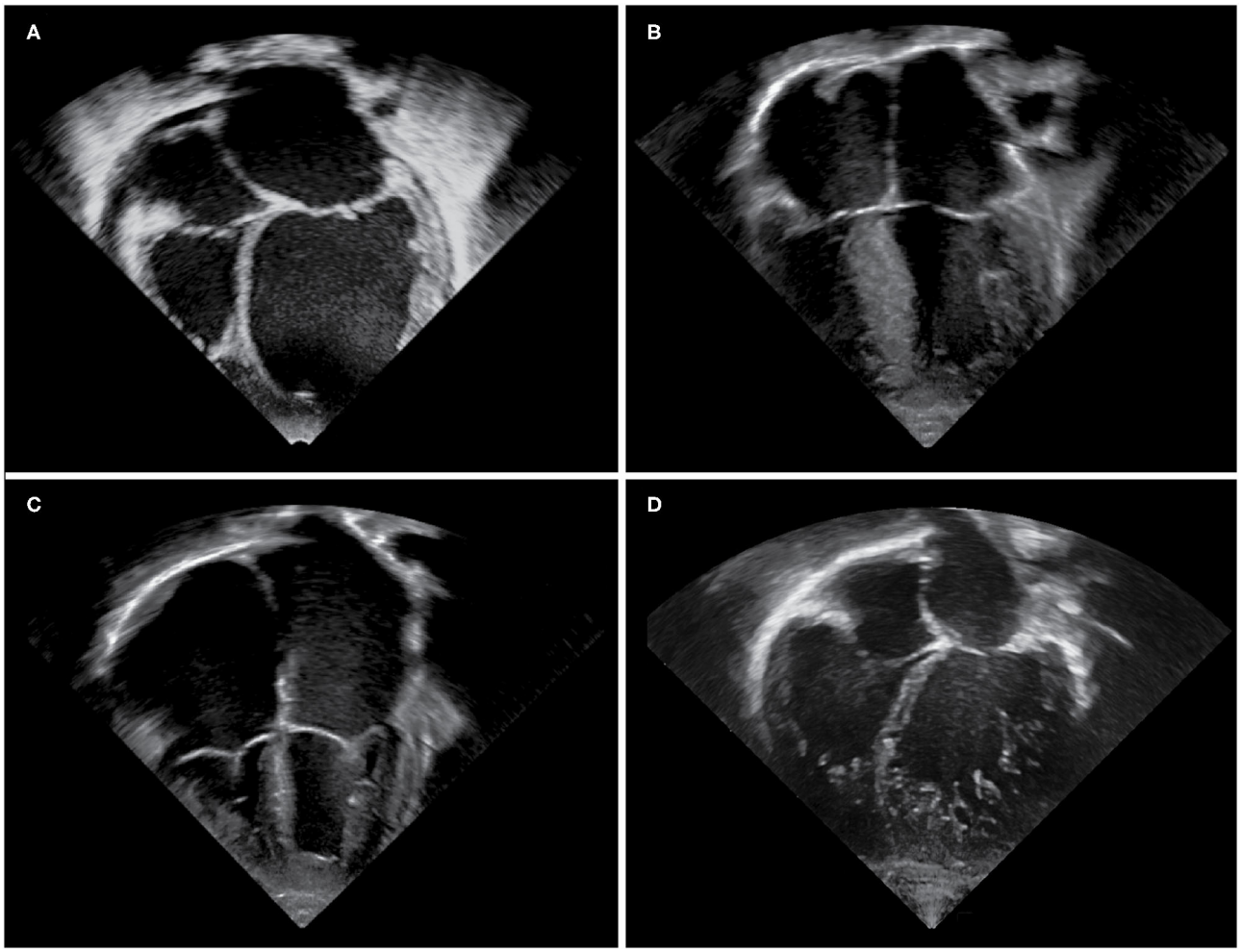

FIGURE 1 | Echocardiographic images of cardiomyopathy phenotypes. Apical four chamber views demonstrating **(A)** a dilated left ventricle and left atrium in a DCM patient, **(B)** hypertrophy of the interventricular septum and left ventricular free wall in a HCM patient, **(C)** massively dilated atria and small right and left ventricular cavities in an RCM patient, **(D)** an extensively trabeculated myocardium with a compacted and non-compacted layer and deep intertrabecular recesses most prominent at the left ventricular apex and free wall in an LVNC patient.

of Cardiology classifies cardiomyopathies according to their predominant phenotype, but does not recognise left ventricular non-compaction as a separate entity, whereas the American Heart Association classifies cardiomyopathies according to their aetiology, with left ventricular non-compaction considered to be a separate entity (25, 26). Some children may present with a mixed phenotype, and cardiomyopathy phenotypes may undulate or transition during the course of disease (1, 12, 13, 27). This review summarises the most common forms of paediatric cardiomyopathies, with a focus on epidemiology and natural history.

DILATED CARDIOMYOPATHY

Aetiologies

Dilated cardiomyopathy (DCM) is characterised by left ventricular dilation and systolic dysfunction. Important aetiologies in childhood include infections, toxic causes (including chemotherapy), genetic mutations, and other causes such as inborn errors of metabolism and neuromuscular disorders (1). Recent advances in genetic diagnostics, including the introduction of next-generation DNA sequencing technologies and extended cardiomyopathy gene panels, have increased the detection rate for pathogenic mutations in adult DCM patients to about 40% (28, 29). Sarcomere gene mutations are thought to be responsible for 35–40% of genetic cases (1). Evidence of myocarditis has been found in up to one third of children with DCM undergoing early endomyocardial biopsy (20, 21, 30). Individuals with variants in genes coding for cardiac structural proteins may be particularly susceptible to severe myocarditis (31, 32). However, despite significant diagnostic progress over the last decade, the aetiology of childhood DCM often remains unknown.

TABLE 1 | Annual incidence and median age at diagnosis for each type of cardiomyopathy (CM).

Type of CM	Age range at diagnosis	Number of cases		Median age at diagnosis	Annual incidence per 100,000 children		References
		All cases	Age at diagnosis <1 y		All cases	Age at diagnosis <1 y	
DCM	0–18 y	239	–	1.8 y	0.58	4.58	PCMR (7)
	0–10 y	184	121	7.5 m	0.73	4.76	NACCS (17)
	0–20 y	56	29	1.1 y	0.34	3.8	Finland study (18)
HCM	0–18 y	196	–	5.9 y	0.47	3.2	PCMR (7)
	0–10 y	80	48	5.7 m	0.32	1.89	NACCS (17)
	0–20 y	40	2	13 y	0.24	0.26	Finland study (18)
RCM	0–10 y	8	0	3 y	0.03	0	NACCS (17)
	0–20 y	6	0	7.2 y	–	0	Finland study (18)
Other[a]	0–18 y	15	–	–	0.04	–	PCMR (7)
Unclassified[b]	0–10 y	42	30	3.8 m	0.17	1.18	NACCS (17)
Total	0–18 y	467	193	–	1.13	8.34	PCMR (7)
	0–10 y	314	199	–	1.24	7.84	NACCS (17)
	0–20 y	107	31	–	0.65	4.1	Finland study (18)

[a] *Includes restrictive and other identified types of CM.*
[b] *Includes LVNC (29 of 42 cases).*

Epidemiology

DCM is the most common type of childhood cardiomyopathy. It comprised about half of all cases in the Paediatric Cardiomyopathy Registry (PCMR), a large multicentre North American study, as well as in the National Australian Childhood Cardiomyopathy Study (NACCS), a population-based cohort study which included children younger than 10 years of age at diagnosis. The overall incidence was 0.58–0.73 per 100,000 children and varied significantly by age (see **Table 1**). In both studies, the highest annual incidence was observed during the first year of life (see **Table 1**) (7, 17). A nationwide cardiomyopathy study from Finland, which included only idiopathic cardiomyopathy cases, demonstrated an overall DCM incidence of 0.34 per 100,000 children up to 20 years and an 11-fold higher incidence during the first year of life (see **Table 1**) (18). A large prospective study undertaken in the United Kingdom and Ireland assessed the incidence of paediatric new-onset heart failure due to heart muscle disease of known and unknown aetiologies. The authors reported an incidence of 0.76 symptomatic DCM cases per 100,000 children under 16 years (19). Lymphocytic myocarditis accounted for 22% of all cases in this study, and comprised 16–36% of DCM cases in the PCMR and NACCS (19–21). Endomyocardial biopsy is not universally performed in newly diagnosed cases of DCM and the presence of positive histological findings for lymphocytic myocarditis decreases rapidly within weeks after presentation, which may lead to an underestimation of inflammatory DCM in children (21, 33).

Presenting Features

The majority of children with DCM, particularly infants, present with varying severity of congestive cardiac failure, with symptoms ranging from feeding difficulties to cardiovascular collapse, and rarely sudden death. Older children with a positive family history and those with neuromuscular disorders or inborn errors of metabolism may be diagnosed as part of routine screening, prior to symptom onset. Lymphocytic myocarditis has a phenotype that may be indistinguishable from idiopathic DCM. A history of a prior viral illness is not always present and may be misleading (34). Data from the PCMR and NACCS showed that at the time of diagnosis, 71–90% of children with DCM had clinical evidence of congestive heart failure (20, 21). In the NACCS DCM cohort, 5% presented with sudden cardiac death, 2% with exercise intolerance or arrhythmia, and 3% for routine family screening. Intensive care unit admission was required in 45% and inotropic support was administered in 40% (21). Significant comorbidities associated with cardiomyopathy related intensive care unit admissions included renal failure, thromboembolic events and hepatic impairment (35).

Natural History

Outcomes for children with DCM are highly variable, ranging from complete recovery to death or requirement for transplantation. Beyond infancy, DCM is the most common indication for heart transplantation in children (36). Data from the PCMR and NACCS demonstrated an overall 5 year transplant-free survival of 54–65 % (see **Table 2**) (20, 21). Risk stratification helps to identify individuals who can be treated medically and those who require advanced heart failure therapy including listing for heart transplantation. Transplant-free survival analysis according to the underling aetiology from the PCMR is shown in **Table 2** (20). Long-term outcome data from the NACCS revealed transplant-free survival of 56% after 20 years (22). The highest risk was observed early after diagnosis, with a 26% risk of death or transplantation within the first year and only 1% per year thereafter (22). Similar results were

TABLE 2 | Transplant-free survival for each type of cardiomyopathy (CM).

Patient characteristics				Transplant-free survival rate after diagnosis			References
Type of CM	**Subgroups**	**Age range at diagnosis *(median age)***	**Number of cases**	**1 y**	**5 y**	**10 y *(>10 y)***	
DCM	All	0–10 y	175	74%	65%	62% *(20 y: 56%)*	NACCS (22)
	All	0–18 y *(1.54 y)*	1,426	69%	54%	46%	PCMR (20)
	Idiopathic		941	61%	47%	42%	
	Myocarditis		222	79%	73%	60%	
	Familial		66	81%	59%	59%	
	IEM		54	84%	78%	78%	
	MFS		15	91%	76%	76%	
	NMD		125	83%	52%	26%	
HCM	All	0–10 y *(0.48 y)*	80	86%	–	80% (*20 y: 78%)*	NACCS (8)
	All	0–18 y	855	–	–	–	PCMR (23)[a]
	Idiopathic	*(7.07 y)*	634	94.4 %	89.8%	85.3%	
	IEM	*(0.42 y)*	74	53.6%	41.7%	–	
	MFS	*(0.41 y)*	77	82.4%	74.4%	74.4%	
	NMD	*(10.10 y)*	64	98.2%	98.2%	91.7%	
	All	0–16 y *(5.2 y)*	687	95.6%	90.6%	86.3%	UK study, Norrish et al. (6)
	Non-syndromic		433	97.6%	92.7%	87.5%	
	RASopathy		126	92.5%	90.5%	85.9%	
	IEM		64	82.2%	66.4%	66.4%	
	FRDA		59	100%	97.1%	97.1%	
RCM	All	0–18 y	152	–	–	–	PCMR (10)
	Pure RCM	*(6.1 y)*	101	48%	22%	–	
	RCM/HCM	*(6.3 y)*	51	65%	43%	–	
LVNC	All	0–10 y *(0.3 y)*	29	69%	52%	48% *(15 y: 45%)*	NACCS (13)
	All	0–14 y *(9.3 y)*	242	–	–	–	USA study, Brescia et al. (57)
	Dilated		46	–	63%	–	
	Hypertrophic		66	–	86%	–	
	Mixed		68	–	64%	–	
	Pure		62	–	98%	–	

IEM, inborn errors of metabolism; MFS, malformation syndromes; NMD,neuromuscular disorders; FRDA, Friedreich's ataxia.
[a]*Numbers represent overall survival rates by aetiologies, transplantation status not further specified.*

found in the UK study of children admitted with new-onset heart failure secondary to dilated cardiomyopathy. Event-free survival at 1 year was only 66 and 16% had undergone heart transplantation within the first year following diagnosis (19).

Other risk factors in children with DCM relate to age at diagnosis and the severity of cardiac dysfunction. Risk factors for death or transplantation in the NACCS comprised age below 4 weeks or above 5 years at presentation, familial dilated cardiomyopathy, a lower initial fractional shortening *z*-score and failure to improve fractional shortening *z*-score during follow-up (21, 22). Favourable outcomes including transplant-free survival and reverse remodelling were more frequent in children with lymphocytic myocarditis compared to other aetiologies, with echocardiographic normalisation of LV function found in 92% during follow-up (22). Although less frequent, reverse modelling has also been observed in children without proven or suspected myocarditis. Everitt et al. reported echocardiographic recovery of LV function within 2 years in 22% of cases with idiopathic DCM included in the PCMR. Younger age (<10 years) and less LV dilation at diagnosis were independent predictive factors of echocardiographic normalisation. Some patients developed recurrence of congestive cardiac failure following initial echocardiographic normalisation (37). Persistence of increased levels of N-terminal proBNP after initial stabilisation was found to be predictive for the risk of cardiac death in children with idiopathic DCM (38).

Pahl et al. reviewed risk factors for sudden cardiac death (SCD) in DCM from the PCMR and found a 5-year incidence of 3%. Younger age at diagnosis (<14.3 y), systolic LV dilation (LV

end systolic dimension z-score >2.6) and posterior wall thinning were identified as risk factors for sudden death (39).

The overall survival for childhood DCM in North America has improved in the most recent era, despite rates of echocardiographic normalisation and cardiac transplantation that did not differ by comparison with a previous era (40). This may be due to improved resuscitation and/or the use of adult-based chronic heart failure therapies.

HYPERTROPHIC CARDIOMYOPATHY

Aetiologies

Hypertrophic Cardiomyopathy (HCM) is a condition characterised by left or biventricular hypertrophy in the absence of structural heart disease or increased ventricular afterload. Sarcomeric mutations represent the most common genetic cause in children and adults with a detection rate of about 60% in childhood HCM diagnosed beyond the first year of life, similar to that of adult HCM patients (41). By comparison with adult HCM, childhood HCM comprises a much more heterogenous group with diverse aetiologies and spectrum of disease (6). Genetic causes include inborn errors of metabolism, malformation syndromes (primarily RASopathies), neuromuscular disease as well as pathogenic mutations in genes coding for sarcomeric proteins. Typical causes within these four categories are Pompe disease, Noonan syndrome, Friedreich ataxia, and MYBPC3 or MYH7 mutations, respectively (1, 23).

Epidemiology

HCM forms the second commonest group of childhood cardiomyopathies, comprising 25–42% of all cases, respectively. The overall annual incidence is 0.24–0.47 per 100,000 children (see **Table 1**) (7, 17, 18). HCM caused by inborn errors of metabolism and malformation syndromes generally presents in infancy and contributes significantly to an early first peak in incidence. A second, smaller peak during adolescence and early adult life is largely due to sarcomeric protein mutations (1, 41). Data from the NACCS, which included children only aged 0–10 year at diagnosis and excluded those with metabolic and neuromuscular conditions, demonstrated a median age of 5.7 months at presentation. The NACCS and PCMR reported a marked decline in incidence between the first year and subsequent years of life (see **Table 1**). In the Finnish study, which also excluded patients with metabolic and neuromuscular aetiologies but included children up until 20 years at diagnosis, the median age at diagnosis was 13 years, and 39% of patients were over 15 years of age at presentation (see **Table 1**).

Presenting Features

The clinical status at presentation ranges from asymptomatic to symptoms of exercise intolerance, chest pain, palpitations, syncope, or cardiac arrest (1). Congestive heart failure or arrhythmic symptoms are found in 10–15% of cases at presentation (6, 24). Children with inborn errors of metabolism and malformation syndromes generally present earlier, and are more likely to have congestive heart failure at the time of diagnosis (23). Aborted sudden cardiac death or out of hospital arrest are an uncommon initial presentation in childhood HCM (6).

Natural History

The natural history and outcome of childhood HCM largely depend on the age at presentation and the underlying aetiology. The highest risk of mortality is seen in those diagnosed during the first year of life (24).

Overall survival free from death or transplantation was found to be about 90% at 5 years and 78% at 20 years from presentation (see **Table 2**) (6, 8, 24). The risk of mortality or transplantation was 14% during the first year after presentation, decreasing to 0.4% per subsequent year (8).

The worst outcomes are in infants with heart failure at the time of diagnosis and in older children with marked restrictive pathophysiology. Other risk factors include concentric left ventricular hypertrophy at diagnosis, Noonan syndrome, and increasing LV ventricular free wall thickness and worsening LV systolic function during follow-up (8). In the PCMR, children with non-syndromic HCM diagnosed before 1 year of age had a higher mortality, which reduced in those surviving infancy (23). Similarly, Noonan syndrome patients had a markedly reduced 1-year survival when diagnosed with congestive heart failure before 6 months of age (42). The highest 5 year survival rate was observed in HCM secondary to neuromuscular disease (see **Table 2**) (23).

While congestive heart failure accounts for the majority of early deaths in childhood HCM, the most common mode of death overall is SCD (6, 8). Arrhythmic events have been observed with a rate of 1.2 per 100 patient years in a large UK study, with more frequent occurrence in non-syndromic patients (6). The identification of specific paediatric risk factors for SCD is essential to guide implantable cardioverter defibrillator (ICD) insertion in a population that frequently experiences no cardiac symptoms in their daily life. A systematic review and meta-analysis of clinical risk factors for sudden cardiac death in childhood cardiomyopathy identified previous adverse cardiac events, non-sustained ventricular tachycardia, syncope, and extreme left ventricular hypertrophy as major factors (43). Norrish et al. recently described a novel risk prediction model for SCD in childhood HCM, with the objective of providing individualised risk estimates. Unexplained syncope, maximal left ventricular wall thickness, left atrial diameter, and non-sustained VT were found to have the strongest association with the composite outcome of SCD or an equivalent event (44). Miron et al. also described an SCD risk prediction model for paediatric HCM. This group used the above mentioned four risk factors as well as age at diagnosis for a clinical model and added the presence of a pathogenic gene variant for a combined clinical/genetic model (45). The relationship between left ventricular outflow obstruction and sudden death is complex, with some studies showing either a protective effect or an inverse relationship in children with the highest gradients (44–46). Arrhythmic events leading to sudden cardiac death continue to occur in adult cohorts at about 0.7% per year (47).

RESTRICTIVE CARDIOMYOPATHY

Aetiologies

Restrictive cardiomyopathy (RCM) is a rare form of heart muscle disease defined by "normal or decreased volume of both ventricles associated with biatrial enlargement, normal left ventricular wall thickness and atrioventricular valves, impaired ventricular filling with restrictive physiology, and normal (or near normal) systolic function" (26). Nearly a quarter of patients with RCM have a family history of cardiomyopathy (10). An increasing number of genetic mutations in sarcomeric and non-sarcomeric proteins have been reported, providing evidence of overlap with other forms of cardiomyopathy (5, 48, 49). A significant subgroup of RCM cases has a mixed phenotype, most commonly combining characteristics of RCM and HCM (10). RCM has also been described in association with inborn errors of metabolism, infiltrative disease, and skeletal myopathy (1, 50).

Epidemiology

RCM is the rarest form of paediatric cardiomyopathy with an incidence of 0.03–0.04 per 100,000 children in Australia and the United States (see **Table 1**) (7, 17). RCM accounted for 2.5% of cases in the NACCS, and the PCMR reported 3% of pure RCM cases and an additional 1.5% of mixed RCM/HCM phenotype cases (7, 17). Age at diagnosis ranges from early infancy to late adulthood (1). Unlike other childhood cardiomyopathies, RCM becomes more frequent with increasing age. Only 10% of pure RCM cases in the PCMR were diagnosed during the first year of life (10).

Presenting Features

Early symptoms of RCM may be non-specific, including general fatigue and exercise intolerance. Clinical findings secondary to elevated systemic and pulmonary venous pressures include peripheral oedema, hepatomegaly, pulmonary oedema, and pulmonary hypertension (1). In the later stages of disease, patients may develop systolic dysfunction (1). Syncope is a non-specific but ominous presenting symptom, which may be caused by arrhythmias, coronary ischemia, or thromboembolic events (51).

Natural History

Whilst RCM has the worst outcomes of any childhood cardiomyopathy, the natural history has largely been obscured by early referral for transplantation. Long-term transplant-free survival data is therefore sparse. Because of its relentless and progressive nature, patients are at risk of sudden death, congestive heart failure, atrial and ventricular arrhythmias, conduction disorders and thromboembolism (1, 52). Transplantation free survival for children with pure RCM in the PCMR was 48 and 22% 1 and 5 years after diagnosis, respectively (10). The mixed phenotype group (RCM/HCM) demonstrated a 2-fold higher 5 year transplant-free survival (see **Table 2**). Overall freedom from death after 5 years was identical for both cohorts, indicating a preference for earlier transplantation in pure RCM patients (10). Russo et al. reviewed 21 cases of RCM in a single centre retrospective analysis, and reported transplantation free survival of 80.5 and 20% at 1 and 10 years, respectively (53). Anderson et al. analysed their institutional experience for children with RCM comparing a historical cohort of 9 cases, diagnosed between 1975 and 1993, with a contemporary cohort of 12 cases. Transplantation free survival over 5 years was 38% in both groups, however overall survival in the contemporary group was 80 vs. 38% in the historical group (54).

Due to the infrequence of childhood RCM and small study cohorts, assessment of risk factors for outcome has proven difficult, and results have been inconsistent. In the PCMR, heart failure symptoms and lower fractional shortening *z*-score at diagnosis were identified as independent risk factors for decreased transplant-free survival (10). Similarly, higher initial echocardiographic left atrial dimensions and a requirement for diuretics during follow-up have been associated with increased mortality (49). Anderson et al. observed an association of marked elevation of mitral valve Doppler E/e' ratio on echocardiography with increased mortality (54). Rivenes et al. evaluated risks factors predictive of sudden death and cardiovascular collapse in 18 children with RCM from a single centre retrospective study. They reported an increased risk of ischemia-related complications and mortality in the entire patient group. The risk of sudden death was highest in girls with clinical signs suggestive of ischemia, in particular chest pain and syncope at presentation. The subgroup at risk of sudden death appeared well and had no clinical evidence of ongoing congestive heart failure (51). Walsh et al. observed that PR prolongation and a wider QRS complex on a baseline ECG were associated with an increased incidence of acute cardiac events, and they found a substantial risk for acute high-grade heart block in RCM patients (52). An elevated pulmonary vascular resistance is present in up to 40% of children with RCM and may impact on the timing for transplant referral (11). Serial cardiac catheterisation is often undertaken to detect this serious complication which may impact on transplant suitability (5, 11).

LEFT VENTRICULAR NON-COMPACTION

Aetiologies

Left ventricular non-compaction (LVNC) is a heterogenous form of cardiomyopathy characterised by excessive trabeculation of the left or both ventricles with deep intertrabecular recesses, most frequently affecting the left ventricular apex. LVNC was classified as a separate cardiomyopathy by the American Heart Association in 2006 (26) however there is ongoing discussion about whether it is a distinct entity or a morphological phenotype (55). Arrest in normal endomyocardial morphogenesis with failure of trabecular compaction is thought to be causative especially in paediatric cases (3). A similar phenotype can manifest at any time in adult life secondary to conditions associated with an increased left ventricular preload (56). LVNC can be isolated or associated with other cardiomyopathy phenotypes, arrhythmias, or congenital heart disease. LVNC is commonly found in Barth syndrome, an X-linked recessive disorder caused by tafazzin gene mutations, and has also been reported in patients with inborn errors of metabolism, neuromuscular diseases, and genetic syndromes (1). Genetic testing detects variants in 30–45% of cases, with sarcomeric mutations found most frequently (3, 56).

Epidemiology

The rates of diagnosis of LVNC in children have increased during the last decades, which is thought to reflect increased awareness and improved imaging techniques rather than a rise in incidence (12). Data from the NACCS demonstrated an incidence of 0.11 per 100,000 children aged 0–10 years, and a 7-fold higher incidence in infants (see **Table 1**). LVNC was found in 9.2% of children diagnosed with cardiomyopathy under 10 years (17). In the PCMR, LVNC was present in 4.8% of cases. LVNC was found to be associated with dilated, hypertrophic and indeterminate phenotypes in 59, 11, and 8% of cases, respectively, and isolated LVNC occurred in the remaining 23% of cases. The median age at diagnosis was significantly higher in isolated LVNC (9.8 years) compared to cases with mixed phenotypes (0.4–0.6 years) (12).

Presenting Features

Patients with LVNC may be found on routine screening but may also present with thromboembolic events, arrhythmias, or congestive heart failure (3). The variability of presenting symptoms reflects the phenotypic diversity, and associated features of other types of cardiomyopathies contribute significantly to the clinical picture. In the largest cohort of paediatric LVNC patients, of which almost 40% were infants, 25% presented primarily with congestive heart failure, 17% with arrhythmias, 19% with a heart murmur, and 37% were asymptomatic (57). A positive family history of cardiomyopathy was present in 23% of all cases, however, only 25% of this subgroup had a family history of LVNC.

Natural History

The outcome of paediatric LVNC is highly variable and depends on the underlying pathophysiology (1). Brescia et al. reviewed the risk of mortality and sudden death in the largest published cohort of paediatric LVNC patients. They found a strong relationship between the identified phenotype and the risk of death or transplantation. Five-year transplant-free survival rate was excellent for the normal-dimension phenotype, intermediate for the hypertrophic phenotype and worst for the dilated and mixed phenotypes (see **Table 2**). The greatest risk factors for death or transplantation were the presence of systolic dysfunction and/ or arrhythmias. Sudden cardiac death occurred in 6.2% of cases over a 19-year study period, with systolic dysfunction present in 95% and documented arrythmia in 60% of these patients. Early presentation during the first year of life was an additional independent risk factor, with a 25% risk of death or transplantation in infantile LVNC (57). Jefferies et al. similarly observed the worst outcome for patients with LVNC and the dilated or indeterminate phenotype in the PCMR. The risk of death was highest in the first year after diagnosis (12). Shi et al. reviewed long-term outcomes of children with LVNC from the NACCS. This cohort included mainly young and severely affected infants, with a median age of 0.3 years at diagnosis and congestive heart failure present in 83% at the time of diagnosis. Freedom from death and transplantation was 45% at 15 years after diagnosis (see **Table 1**). Propensity score matching suggested a 2-fold higher risk of death and transplantation for patients with LVNC and a dilated phenotype compared to children with DCM from the same registry (13). Children with an isolated LVNC phenotype and without observable cardiac dysfunction have been found to have a favourable outcome (12). However, progression to an associated cardiomyopathy phenotype with a risk of mortality has been observed in a small proportion of cases, therefore ongoing surveillance is recommended (12).

DISCUSSION

Over the last 2 decades, registries and national studies have provided important data on epidemiology and outcomes of childhood cardiomyopathies. The reported overall annual incidence was between 0.65 and 1.24 per 100,000 children (7, 17, 18). Consistently throughout these epidemiological studies, the annual incidence of cardiomyopathy during the first year of life was 6–7 times higher than the above-mentioned average incidences (7, 17, 18). This peak incidence during infancy was found in all types of cardiomyopathy except for RCM which typically presents later in childhood (7, 17, 18). PCMR data demonstrated a small second peak during adolescence which was related to HCM and cardiomyopathies secondary to neuromuscular diseases (7).

LVNC cardiomyopathy has increasingly gained attention over the last two decades and there is ongoing discussion with regards to its diagnostic criteria (25, 26). Increasing rates of diagnosis reflect an increasing awareness of this entity compared to prior eras (12).

While there has been a dramatic improvement in survival of congenital heart disease over the last decades, the overall outcomes for childhood cardiomyopathies remain unfavourable (58, 59). Recovery from inflammatory cardiomyopathies or successful rhythm control in arrhythmogenic heart failure represent exemptions. Childhood cardiomyopathies comprise the most common indication for cardiac transplantation beyond the first year of age (36). The highest risk of death or cardiac transplantation occurs during the first year after diagnosis (19, 22). Knowledge of the natural history and risk factors for adverse outcomes, gained from registries and large multicentre studies, assists in risk stratification and case selection for advanced heart failure therapies, and for ICD insertion for prevention of sudden cardiac death.

CONCLUSION

Paediatric cardiomyopathies, although rare, carry a substantial burden of disease due to the risk of morbidity and mortality and a lack of curative therapy. Established registries have provided valuable insights into the natural history and risk factors, assisting in decision-making on sudden cardiac death prevention and cardiac transplantation.

AUTHOR CONTRIBUTIONS

AR drafted the manuscript and designed figures and tables. RW supervised the project and contributed substantially to the final version of the manuscript. Both authors contributed to the article and approved the submitted version.

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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 ≤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.

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

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.

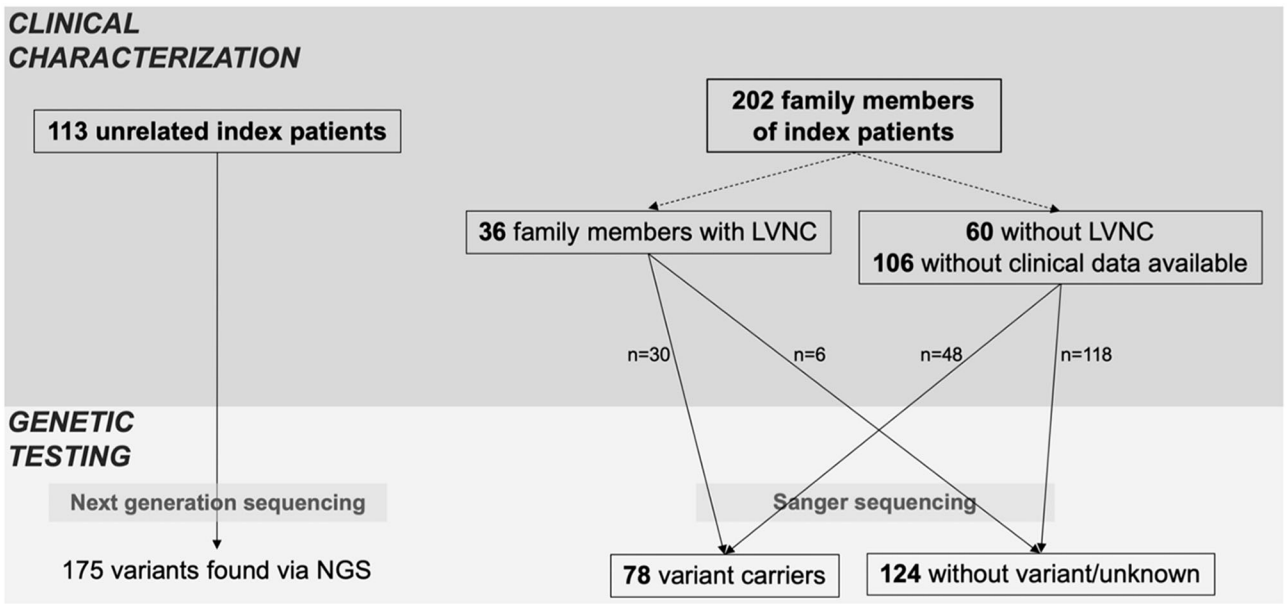

FIGURE 1 | Index patients and family screening. Overview of clinical characterization and genetic testing of patients, including all available family members. Family members were classified as variant carriers, when they carried at least one of the variants of the respective index patient.

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

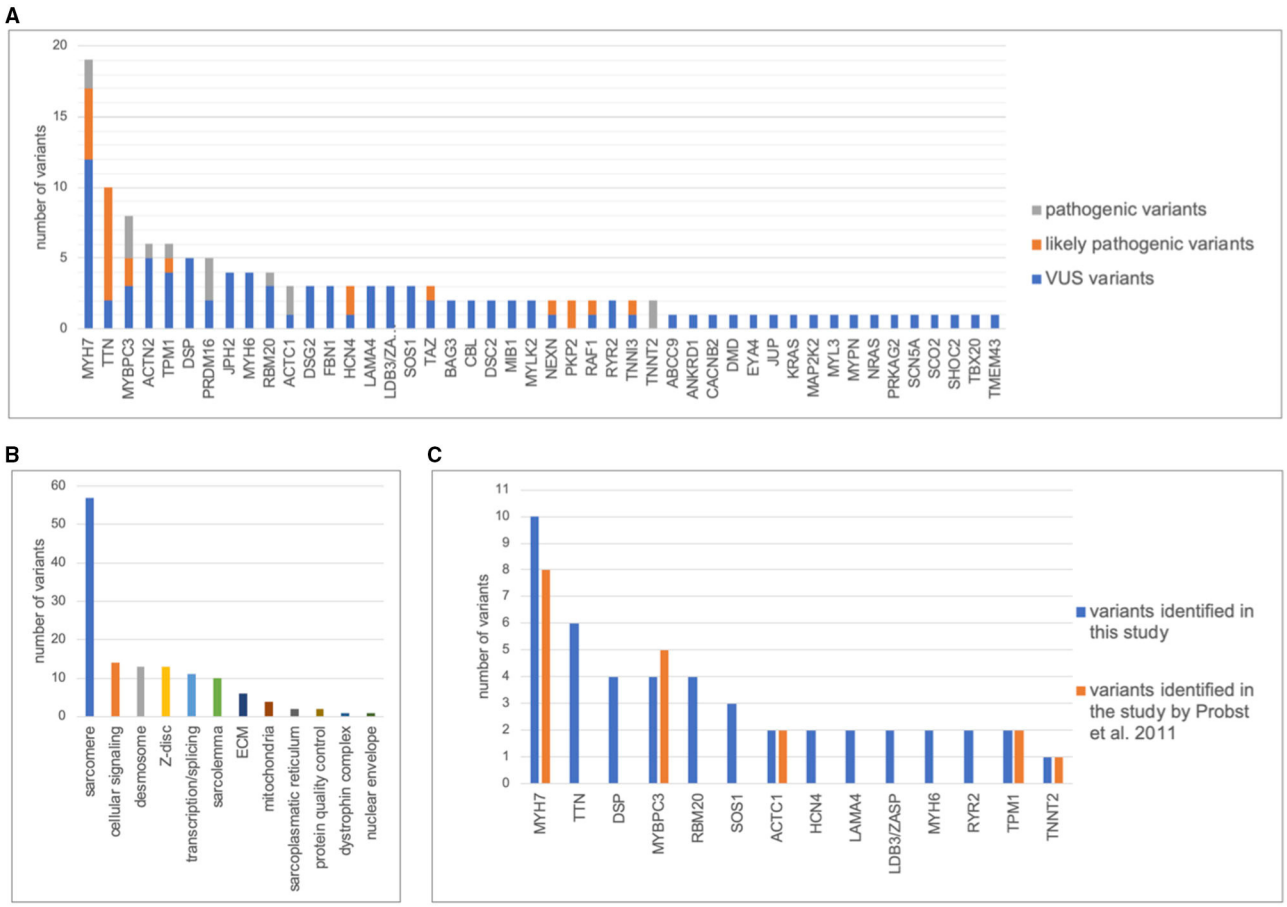

FIGURE 2 | Distribution of genetic variants. Distribution of variants in cardiomyopathy genes including pathogenic, likely pathogenic, and variants of unknown significance (VUS). The figure only contains the most frequent genes. **(A)**, Number of variants detected per gene. **(B)**, Distribution of 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**.

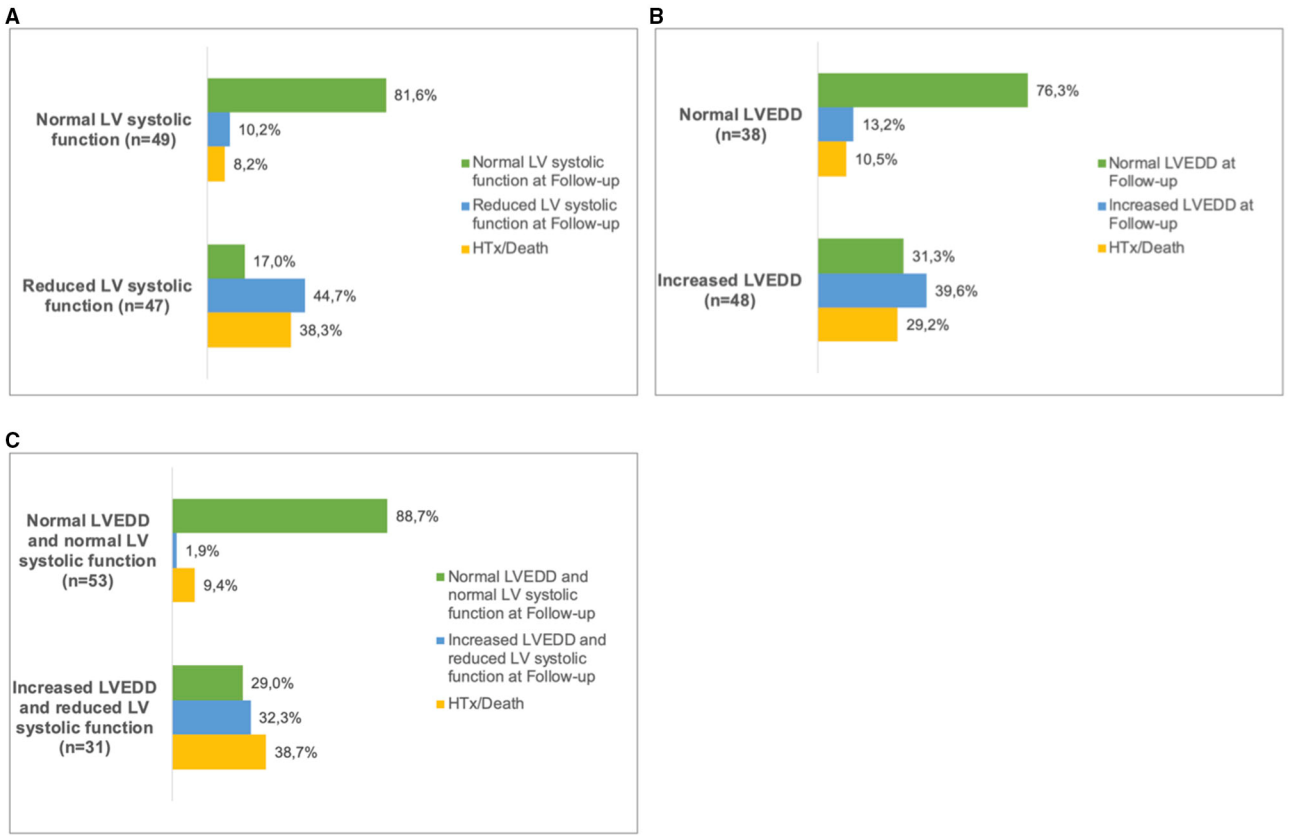

FIGURE 3 | Follow up in LVNC patients. Patients were sorted into subgroups according to their phenotype at first presentation. **(A)**, normal LV systolic function and reduced LV systolic function. **(B)**, normal LVEDD and increased LVEDD and **(C)**, 'normal LVEDD and normal LV systolic function' and '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.

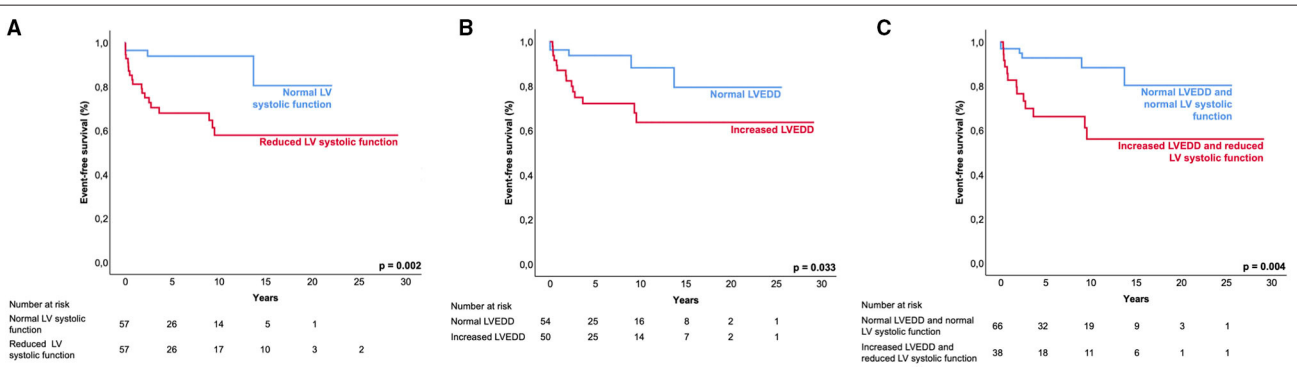

FIGURE 4 | Event-free survival of LVNC patients. Kaplan-Meier analysis shows the event-free survival to the combined endpoint of mechanical circulatory support, heart transplantation, survived sudden cardiac death, and all-cause death. Event-free survival between patient groups. **(A)**, normal LV systolic 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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SYSTEMATIC REVIEW
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Arrhythmogenic Right Ventricular Cardiomyopathy in Pediatric Patients: An Important but Underrecognized Clinical Entity

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy characterized by fibrofatty infiltration of predominantly the right ventricular (RV) myocardium. Affected patients typically present as young adults with hemodynamically stable ventricular tachycardia, although pediatric cases are increasingly recognized. These young subjects often have a more severe phenotype with a high risk of sudden cardiac death (SCD) and progression toward heart failure. Diagnosis of ARVC is made by combining multiple sources of information as prescribed by the consensus-based Task Force Criteria. The description of Naxos disease, a fully penetrant autosomal recessive disorder that is associated with ARVC and a cutaneous phenotype of palmoplantar keratoderma and wooly hair facilitated the identification of the genetic cause of ARVC. At present, approximately 60% of patients are found to carry a pathogenic variant in one of five genes associated with the cardiac desmosome. The incomplete penetrance and variable expressivity of these variants however implies an important role for environmental factors, of which participation in endurance exercise is a strong risk factor. Since there currently is no definite cure for ARVC, disease management is directed toward symptom reduction, delay of disease progression, and prevention of SCD. This clinically focused review describes the spectrum of ARVC among children and adolescents, the genetic architecture underlying this disease, the cardio-cutaneous syndromes that led to its identification, and current diagnostic and therapeutic strategies in pediatric ARVC subjects.

Keywords: arrhythmogenic (right ventricular) cardiomyopathy, natural history, management, children, adolescent, pediatric, naxos disease

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INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a heritable condition of the heart and is part of the phenotypic spectrum of arrhythmogenic cardiomyopathies (ACM) that includes right-dominant (ARVC), left-dominant (ALVC) and biventricular arrhythmogenic cardiomyopathy (**Figure 1**) (1, 2). Our manuscript focuses on the right-dominant subform of pediatric ACM (i.e.,

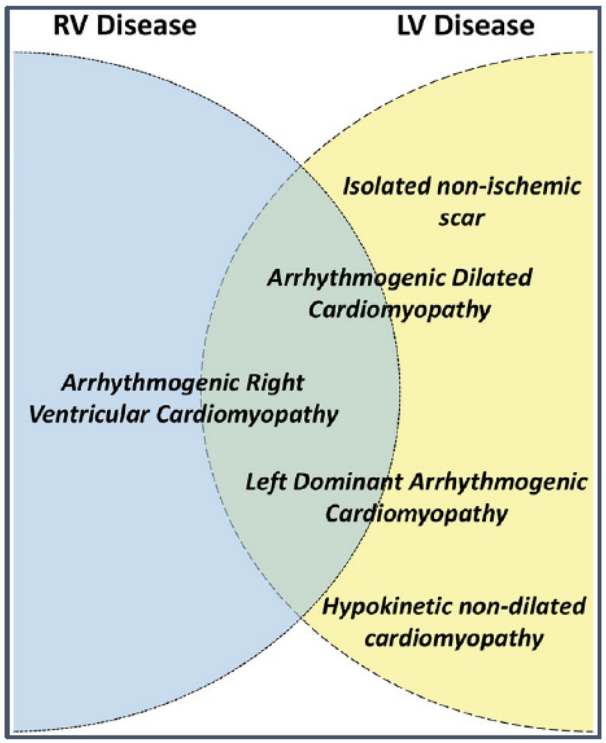

FIGURE 1 | Terms used to describe different arrhythmogenic cardiomyopathy phenotypes and their possible relationship to left (LV) and right ventricular (RV) disease. Source: (1), obtained with permission.

ARVC); a description of left-dominant and biventricular ACM in pediatric patients can be found in the same issue of this journal (reference). ARVC is characterized by potentially severe ventricular arrhythmias and structural alterations of the ventricular myocardium, which are identified by macro- and microscopic pathological examination and/or abnormal cardiac imaging (1). While right ventricular (RV) structural abnormalities have been predominantly reported, advanced imaging techniques reveal increased incidence of left ventricular (LV) or biventricular involvement (3–5). A sudden death that occurred during exercise in a young doctor, who was previously noted to have episodes of sustained ventricular tachycardia (VT) of RV origin, was the "case 0" of the fundamental report of ARVC as a cause of juvenile sudden death (6, 7). Indeed, although the estimated prevalence of ARVC in the general population is only 1:5,000, it represents one of the most common causes of juvenile sudden death (8).

Since the initial disease description by Marcus and colleagues in 1982, most studied ARVC cohorts constituted mainly of adults (3). Arrhythmia-related episodes characterize affected patients, who commonly present at late adolescence or young adulthood. In subsequent years, familial occurrence of ARVC was recognized (9, 10), and pediatric cases were increasingly reported. These young patients often present with a high risk of severe ventricular arrhythmias or sudden cardiac death (11), underscoring the need for early disease detection. Nonetheless, reduced penetrance and variable expressivity in familial ARVC, complicated linkage studies during the early genotyping attempts (7). A breakthrough came with the identification of Naxos syndrome, a fully penetrant autosomal recessive disorder associating ARVC with a cutaneous phenotype of palmoplantar keratoderma and wooly hair that characterizes the patient from infancy. A pathogenic variant in the desmosomal protein junctional plakoglobin (*JUP*) was identified in Naxos disease and was the first causative gene for ARVC (12). Subsequently, pathogenic variants in other desmosomal proteins were related to ARVC. As a result, ARVC is currently considered a heritable desmosomal disorder and more than 60% of probands are now found to harbor a pathogenic desmosomal variant. Yet, non-desmosomal genes are increasingly identified among those with biventricular or left-dominant disease (13).

Although disease evolution and prognosis have been studied in large cohorts of index patients and family members (14), the question of when and how the disease manifests in children has not yet been elucidated. Few cohorts and sporadic cases of children with ARVC have been reported. A systematic follow-up of asymptomatic children carrying a pathogenic genetic variant will provide important information on disease initiation and uncover the earliest signs on how ARVC evolves during childhood.

This clinically focused review describes the spectrum of ARVC among children and adolescents, the genetic architecture underlying this disease, and the current diagnostic and therapeutic strategies in pediatric ARVC subjects.

DIAGNOSIS

The diagnosis of ARVC may be challenging, as no single modality is sufficiently sensitive or specific to serve as the gold standard for ARVC diagnosis. As such, multiple sources of diagnostic information are combined in a complex set of criteria determined by a Task Force in 1994, which were subsequently modified in 2010 (15). These so-called "Task Force Criteria" (TFC) include major and minor criteria encompassing structural, histologic, electrocardiographic (i.e., depolarization and repolarization), arrhythmic, and family history findings (**Table 1**). Quantitative TFC criteria were derived from a comparison of 108 ARVC probands to healthy controls, in which cut-offs for major criteria were chosen to achieve 95% specificity. Cut-offs with high specificity invariably result in lower sensitivity, which ranged from 17–58% in a recent validation study (16). In contrast, minor criteria have higher sensitivity (up to 82%) but consequently lower specificity (as low as 67%) (16). Overall, 2 major, 1 major and 2 minor, or 4 minor criteria are required for diagnosis.

For those involved in the care of pediatric ARVC patients, it is important to recognize that the 2010 TFC were derived using a predominantly adult population (mean 38 $\pm$ 13 years of age): in fact, only 9 of 108 (8%) probands in the original TFC document were diagnosed between 12 and 18 years of age (15). As such, extrapolation of the TFC for use in pediatric cases should be considered experimental (17). The most important concern for TFC implementation during childhood probably rises for

TABLE 1 | Diagnostic Task Force Criteria for arrhythmogenic right ventricular cardiomyopathy.

I. Structural/functional assessment	
Major	**2D Echocardiography:** • Regional RV akinesia, dyskinesia, or aneurysm • *and* 1 of the following at end diastole: ◦ PLAX RVOT ≥ 32 mm or PLAX/BSA ≥ 19 mm/m^2 ◦ PSAX RVOT ≥ 36 mm or PSAX/BSA ≥ 21 mm/m^2 ◦ Fractional area change ≤ 33% **CMR:** • Regional RV akinesia or dyskinesia or dyssynchronous contraction • *and* 1 of the following: ◦ RV EDV/BSA ≥ 110 mL/m^2 (male) or ≥ 100 mL/m^2 (female) ◦ RVEF ≤ 40% **RV angiography:** Regional RV akinesia, dyskinesia, or aneurysm
Minor	**2D Echocardiography:** • Regional RV akinesia, dyskinesia, or aneurysm • *and* 1 of the following at end diastole: ◦ PLAX RVOT ≥ 29 mm or PLAX/BSA ≥ 16 mm/m^2 ◦ PSAX RVOT ≥ 32 mm or PSAX/BSA ≥ 18 mm/m^2 ◦ Fractional area change ≤ 40% **CMR:** • Regional RV akinesia or dyskinesia or dyssynchronous contraction • *and* 1 of the following (end diastole): ◦ RV EDV/BSA ≥ 100 mL/m^2 (male) or ≥ 90 mL/m^2 (female) ◦ RVEF ≤ 45%
II. Tissue characterization	
Major	Residual myocytes <60% by morphometric analysis (or <50% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy.
Minor	Residual myocytes 60–75% by morphometric analysis (or 50–65% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy.
III. Repolarization abnormalities	
Major	Inverted T-waves in leads V1, V2, and V3 or beyond, in individuals >14 years of age (in absence of complete RBBB QRS ≥120 ms).
Minor	• Inverted T-waves in leads V1 and V2, in individuals >14 years of age (in absence of complete RBBB) or in V4, V5, or V6. • Inverted T-waves in leads V1, V2, V3, and V4 in individuals >14 years of age in the presence of complete RBBB.
IV. Depolarization abnormalities	
Major	Epsilon wave (reproducible low-amplitude signals between end of QRS complete to onset of the T-wave) in V1–3.
Minor	• Late potentials by SAECG in ≥1 of 3 parameters in absence of a QRS of ≥110 ms on standard ECG: ◦ Filtered QRS duration ≥114 ms ◦ Duration of terminal QRS <40 μV ≥38 ms ◦ Root-mean-square voltage of terminal 40 ms ≤ 20 μV • Terminal activation duration of QRS ≥55 ms, measured from the nadir of the S-wave to the end of the QRS, including R', in V1, V2, or V3, in absence of complete RBBB.
V. Arrhythmias	
Major	Non-sustained or sustained VT of LBBB morphology with superior axis.
Minor	• Non-sustained or sustained VT of RVOT configuration, LBBB morphology with inferior axis or with unknown axis. • >500 PVCs per 24 h on Holter monitoring
VI. Family history	
Major	• First-degree relative with ARVC confirmed by TFC • First-degree relative with ARVC confirmed pathologically at autopsy or surgery • Identification of a pathogenic variant categorized as associated or probably associated with ARVC in the patient under evaluation
Minor	• First-degree relative with ARVC history not possible to confirm by TFC • First-degree relative with SCD <35 years of age due to suspected ARVC • Second-degree relative with ARVC confirmed by TFC or pathologically

ARVC, arrhythmogenic right ventricular cardiomyopathy; BSA, body surface area; CMR, cardiac magnetic resonance; LBBB, left bundle branch block; EDV, end-diastolic volume; PVC, premature ventricular complex; RBBB, right bundle branch block; PLAX, parasternal long axis; PSAX, parasternal short axis; RV, right ventricular; RVEF, right ventricular ejection fraction; RVOT, right ventricular outflow tract; SAECG, signal-averaged electrocardiogram; SCD, sudden cardiac death; TFC, Task Force Criteria; VT, ventricular tachycardia.

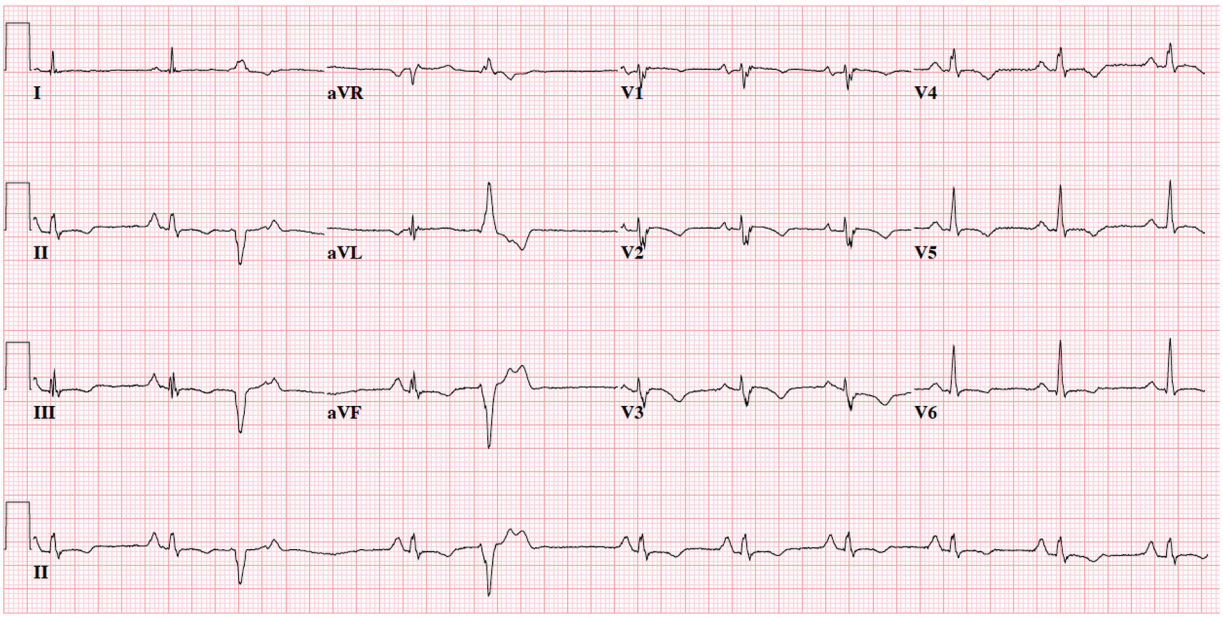

FIGURE 2 | 12-Lead electrocardiogram of 19-year old female with advanced ARVC. Note normal sinus rhythm with frequent premature ventricular complexes with superior axis morphology, voltage criteria for right atrial enlargement, borderline prolonged QRS duration (QRS 0.10s) with fractionated upstroke in V1-3 ("terminal activation duration" >55 ms), and negative T-waves in V1-6 and inferior leads. This patient would fulfill 1 major (T-wave inversion V1–V3 and beyond) and 1 minor criterion (prolonged terminal activation duration) for ARVC. ECG settings: 25 mm/mV; 10 mm/mV; 150 Hz.

ECG criteria: it is widely accepted that right precordial T-wave inversion V1-3 (major criterion for ARVC diagnosis) is a normal finding in children before puberty (18), and therefore the TFC disregard T-wave inversion as diagnostic criterion prior to the age of 14 years. In addition, epsilon waves (also a major criterion for ARVC diagnosis) are often seen in advanced ARVC cases and are likely to be extremely rare in children and adolescents (19). This potentially results in two major criteria with very little (if any) utility in pediatric patients. A typical ECG for an ARVC patient is shown in **Figure 2**. Another equally important concern is that echocardiographic and cardiac magnetic resonance (CMR) criteria are not validated in children. Indeed, the revised TFC determined imaging cut-offs based on a cohort with a mean age of 60 years. Since that time, several reports showed that RV end-diastolic volume decreases on average 4% per decade in healthy subjects, which is not accounted for by adjusting for body surface area (20). This suggests that the TFC may be too sensitive in pediatric patients. A typical CMR for an ARVC patient is shown in **Figure 3**.

Regardless of these limitations, several studies have evaluated the performance of the revised TFC in pediatric or adolescent cohorts. In 2015, Etoom et al. showed that conventional CMR criteria were the strongest contributor to TFC fulfillment in children with ARVC, with almost half of the study population relying on CMR criteria for diagnosis (21). While fatty infiltration and delayed enhancement were rare in this Canadian cohort, a subsequent case series by Slesnick et al. showed that CMR can be highly valuable in determining fibrofatty replacement in pediatric ARVC subjects (22). Similar findings were observed by Steinmetz et al. who showed that any TFC fulfillment on imaging was associated with definite ARVC diagnosis in children with an odds ratio of 8.68 (23). While these studies suggest an important role for imaging in pediatric ARVC evaluation,

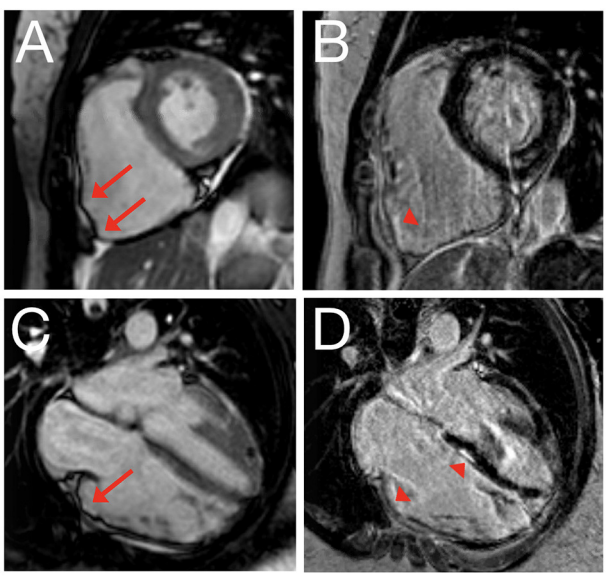

FIGURE 3 | Cardiac magnetic resonance imaging of 22-year old female with ARVC. Still image of cine clip for short axis **(A)** and long axis **(C)** view, with corresponding delayed enhancement images obtained using phase-sensitive inversion recovery in short axis **(B)** and long axis **(D)** view. Red arrows denote dyskinetic segments with outward bulging in systole; arrowheads denote corresponding delayed enhancement.

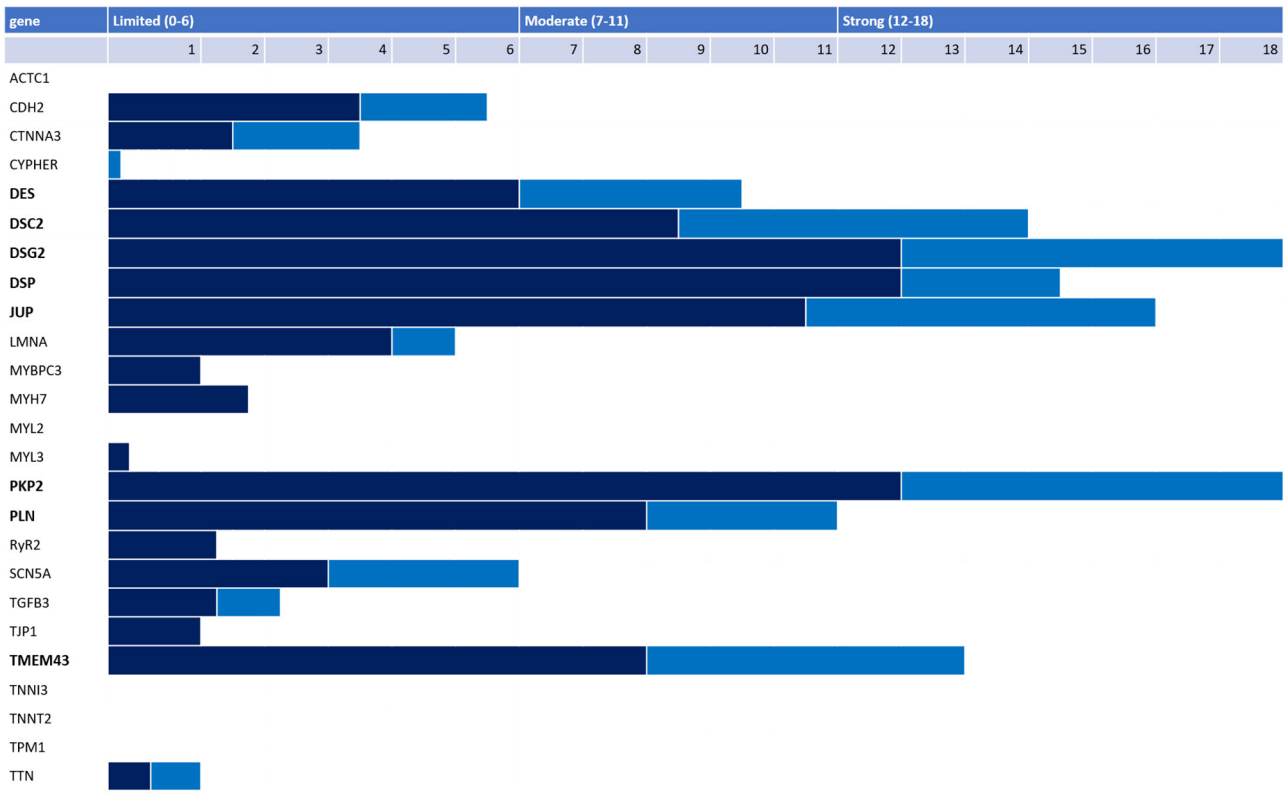

FIGURE 4 | Adjudication of 26 genes reported as ARVC-causing in the literature by an international expert panel. Genetic (dark blue) and experimental (light blue) evidence and final classification 26 genes reported in the literature as associated with ARVC per the ClinGen framework. Only 8 genes had strong or moderate evidence for ARVC causality (bold).

the relatively low prevalence of imaging criteria (particularly using echocardiography) and possibly false-positive results in an important subset of patients are cause for concern (24). Several authors have therefore called for the determination of pediatric-specific diagnostic criteria (25, 26). Until the present time, however, the 2010 TFC remain the clinical gold standard for ARVC evaluation in childhood and adolescence. This seems an appropriate decision, as the pathognomonic criteria for ARVC diagnosis in adults are also valid for young people (27), and the clinical characteristics and outcomes are similar between pediatric and adult patients with a definite ARVC diagnosis as per TFC (11).

GENETICS

Genetic Architecture of ARVC

As described above, ARVC is conventionally considered a disease of the cardiac desmosome with autosomal dominant inheritance and age-related reduced penetrance. Pathogenicity of genetic variants in ARVC evaluation is typically attributed based on the American Society of Clinical Genetics (ACMG) criteria; where class 4 (likely pathogenic) and class 5 (pathogenic) variants are considered to be associated with disease (28). In contemporary cohorts, up to two-thirds of patients with definite ARVC per the 2010 TFC have a pathogenic or likely pathogenic (P/LP) variant in one of 5 genes encoding cardiac desmosome proteins (*PKP2*, *DSP*, *DSG2*, *DSP*, and *JUP*) (14, 29). Well-characterized variants in *TMEM43* and *PLN* also contribute to ARVC, particularly in specific geographic populations (30, 31). Heterozygous truncating and splice P/LP variants in *PKP2* are the most common genetic cause of ARVC (29). *DSP*, *DSG2*, and *PLN* variants are seen more frequently in those with biventricular or left-dominant disease (30, 32–34).

Numerous additional ARVC genes have also been proposed, with uneven quantity and quality of evidence underpinning the ARVC-gene association. Recently, a panel of international experts conducted a rigorous reappraisal of 26 ARVC genes reported in the literature using the semi-quantitative ClinGen framework and found only eight genes had definitive (*PKP2*, *DSP*, *DSG2*, *DSC2*, *JUP*, *TMEM43)* or moderate (*PLN*, *DES*) evidence for causing ARVC (**Figure 4**) (35). Notably, *RYR2* was disqualified as an ARVC gene with the investigators reporting that patients and model systems in the literature had catecholaminergic polymorphic ventricular tachycardia (CPVT), not ARVC. Some genes with only limited evidence are relatively newly characterized and more evidence of ARVC causality may accrue over time. Nonetheless, based on current evidence available, the authors suggested that P/LP variants in only *PKP2*,

DSP, *DSG2*, *DSC2*, *JUP*, *TMEM43*, *PLN*, and *DES* should be used to assign a major genetics criterion when applying the 2010 TFC. Ensuring only genes with substantial evidence of ARVC causality are used to assign the major genetics criterion in the TFC is particularly important for pediatric patients being evaluated for ARVC: they may rely more on this criterion for diagnosis as the TFC disregard right precordial T-wave inversion as a diagnostic criterion before age 14 (18).

Young ARVC patients are particularly likely to harbor one or more P/LP variants. In a cohort of American and Dutch pediatric patients (defined as diagnosis before age 18 or symptomatic presentation of a proband before age 18), 80% had a P/LP variant, significantly more than the 60% of 427 patients presenting as adults (11). Similarly, in a combined cohort of pediatric patients with classic ARVC, biventricular disease, and left-dominant arrhythmogenic cardiomyopathy, 75% of patients with ARVC had at least one P/LP desmosomal variant (36). In the aggregate, genotype positive patients (e.g., harboring one or more P/LP variants) present symptomatically 4 years earlier than gene-elusive probands (14).

Genotype-Phenotype Associations

Broadly, gene-elusive ARVC patients have a similar clinical course to patients with a single P/LP desmosomal variant: a similar proportion develop symptoms, have similar survival free from sustained ventricular arrhythmias, or require transplant (14). Nonetheless, useful data linking genotype to clinical outcomes is emerging. Of particular importance in pediatrics, patients with more than one P/LP variant (including homozygous, compound heterozygous, or di- and tri-genic variants) have younger onset, worse clinical outcomes, and may have an atypical phenotype (37). In a combined US and Dutch cohort of 577 patients with P/LP variants in the desmosomal genes, *TMEM43*, or *PLN*, the 4% of patients with more than one variant had significantly earlier occurrence of sustained VT (mean age 28 $\pm$ 12 years), worse survival free from a first sustained ventricular arrhythmia, and more frequent LV dysfunction (29%), heart failure (19%) and cardiac transplantation (9%) when compared with those with only one P/LP variant (32). In a cohort of 32 ARVC patients with pediatric onset described by DeWitt et al., multiple potentially pathogenic variants (P/LP and variants of uncertain significance [VUS]) were identified in 9 (28%) patients including 6/9 (67%) with biventricular disease (36). Notably, the presence of multiple P/LP *PKP2* variants with at least one on each allele (homozygous or compound heterozygous) seems to herald poor outcomes including pediatric sudden cardiac death and transplant at a young age (36, 38, 39). The presence of loss of function *PKP2* variants on each allele (homozygous or compound heterozygous null alleles) may be particularly devastating. There are multiple reports of infants/fetuses with congenital heart disease and poor neonatal outcomes with this genotype (40, 41), consistent with the requirement of plakophilin-2 for cardiac development (42).

Investigators have considered whether genotype is a useful predictor of arrhythmic risk in ARVC, but with only limited success. As noted above, patients with more than one P/LP variant have worse arrhythmic outcomes. Various papers have suggested that particular genes are associated with higher arrhythmic risk, but most of these assertions have not been replicated. Furthermore, in two statistical models developed recently for individualized risk prediction for incident sustained ventricular arrhythmias and rapid sustained ventricular arrhythmias for ARVC patients, genotype was not a significant predictor and is therefore not included in either model (www.arvcrisk.com) (43, 44). This is notable especially because the multicenter international cohorts included to derive the models were the largest cohort of ARVC patients ever assembled. One exception to the limited utility of genotype in predicting arrhythmic risk is the *TMEM43* c.1073C>T; p.Ser358Leu variant which was initially identified in a large number of patients and families from Newfoundland. This variant is associated with a highly penetrant and arrhythmogenic subtype of ARVC (45, 46). Risk of sudden cardiac death from ventricular arrhythmias is particularly high in males. As such, the literature suggests that genotype predicts arrhythmic risk in ARVC less well than demographic and clinical predictors.

In contrast, there is substantial evidence that the extent of LV involvement and heart failure is associated with genotype. A large study in 577 ARVC probands and family members with P/LP variants from North America and The Netherlands showed that prevalence of left ventricular dysfunction and heart failure varied substantially by genotype (32). LV dysfunction (defined as LV ejection fraction <55%) was seen in 78 (14%) patients while 28 (5%) experienced clinically-recognized congestive heart failure during follow-up. As shown in **Figure 5**, *PKP2* carriers were least likely to have LV dysfunction (9%), whereas those with a P/LP *DSP* variant had significantly more frequent LV dysfunction (40%) and heart failure (13%). *PLN* variant carriers presented at a significantly older age yet had worse long-term prognosis, with more LV dysfunction and heart failure. The observation that P/LP variants in *DSP* and *PLN* disproportionately affect the LV has been replicated in numerous studies including in pediatric patients with *DSP* variants (36). Furthermore, as discussed earlier, multiple P/LP variants heralded worse outcomes, tripling of the prevalence of heart failure (32). The latter was also found in a British study, in which four of eight patients who underwent transplantation or died due to heart failure had multiple genetic variants (47). It is also important to realize that the influence of founder variants is significant: for example, the homozygous p.F531C variant in *DSG2* was associated with a fully penetrant heart failure phenotype in a Chinese cohort (34), while the *TMEM43* p.S358L variant in addition to conveying a high risk of sudden cardiac death is associated with LV dysfunction (31).

Finally, there is increasing evidence that ARVC presenting with chest pain and myocardial enzyme release in the setting of normal coronary arteries ('hot phase') is particularly common in patients with DSP P/LP variants (48–50). These myocarditis-like episodes of acute myocardial injury are particularly common in pediatric ARVC, may recur, and can be familial. Therefore, recurrent myocarditis in a child, or pediatric myocarditis in the setting of a concerning family history should prompt consideration for genetic testing and for evaluation for ARVC.

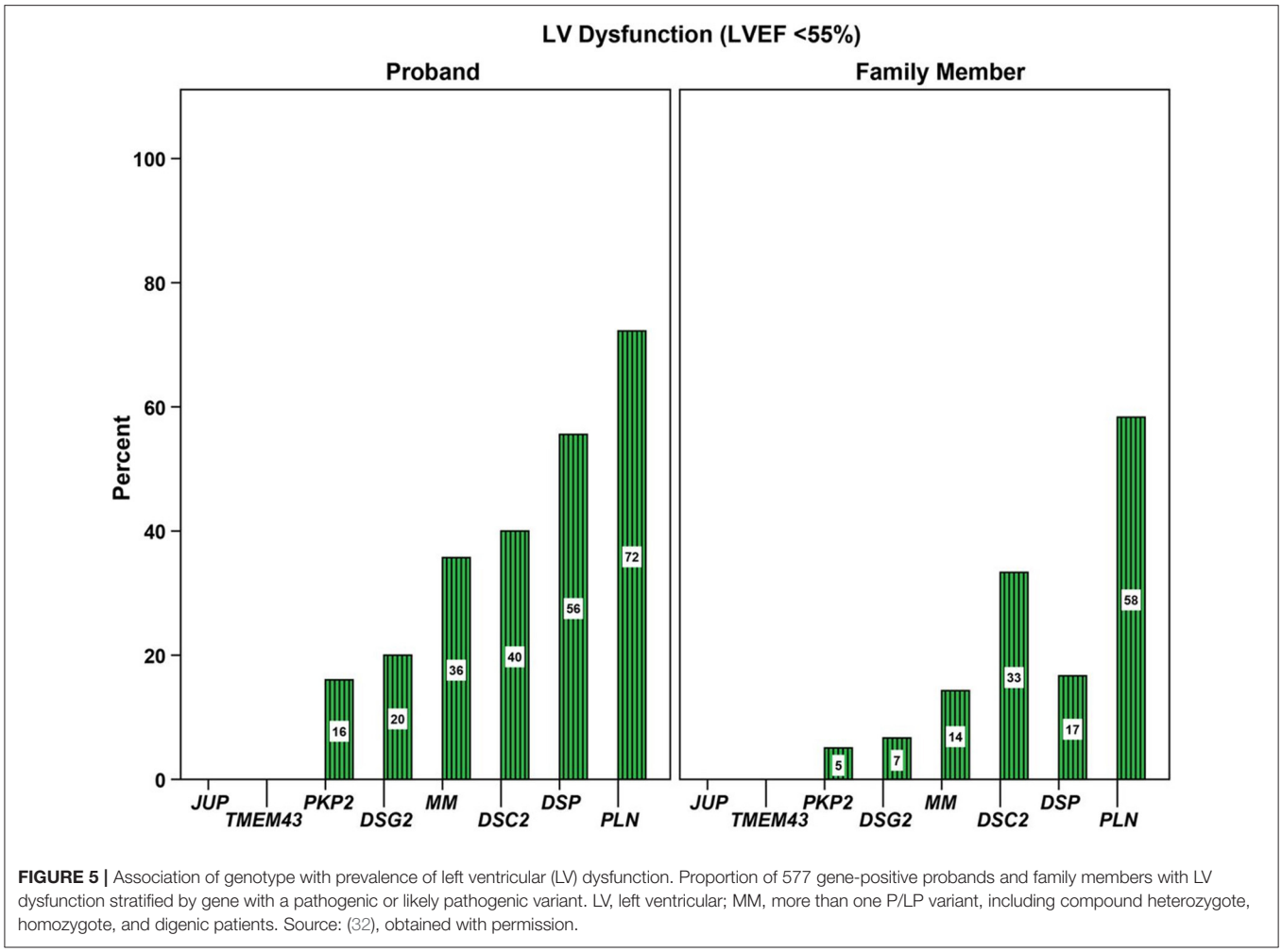

FIGURE 5 | Association of genotype with prevalence of left ventricular (LV) dysfunction. Proportion of 577 gene-positive probands and family members with LV dysfunction stratified by gene with a pathogenic or likely pathogenic variant. LV, left ventricular; MM, more than one P/LP variant, including compound heterozygote, homozygote, and digenic patients. Source: (32), obtained with permission.

Genetic Testing

Genetic testing is recommended for patients with ARVC to confirm the diagnosis, inform management, and inform cascade testing of the family (2, 51). More information on cascade genetic testing can be found below. A multidisciplinary team approach including cardiology, pathology, genetics, and genetic counseling is recommended and valued by patients (2). Best practices for genetic testing for ARVC have been reviewed elsewhere (2, 52), but several aspects that are particularly relevant to the pediatric population are described below and summarized in **Table 2**.

LESSONS LEARNED FROM NAXOS DISEASE

It was as early as 1986 when the first observation of an ARVC phenotype associated with palmoplantar keratoderma (PPK) and wooly hair (WH) was published. The syndrome was named "Naxos disease" after the Aegean island where it was initially observed, and it was reported under this name in the World Congress on Cardiomyopathies in Warsaw in 1993.

Over the years that followed, Naxos disease was mapped to chromosome 17q21 and the causative pathogenic variant was identified: a 2 base-pair deletion in the desmosomal *JUP* gene (*Pk2157del2*TG), truncating the C-terminal of the protein (53). A decade after the first publication, a similar phenotype was reported by Rao et al. and by Carvajal et al. in patients from India (1996) and Ecuador (1998), respectively (54). The cutaneous phenotype consisting of PPK and WH seemed identical to that of Naxos syndrome, while the cardiomyopathy presented with predominant LV involvement diagnosed as dilated cardiomyopathy. Molecular genetic investigations in the families from Ecuador revealed a recessive variant in *DSP* (55). In a later report on an Ecuadorian patient, the name of Carvajal syndrome was used for the first time (56). It is worth mentioning, however, that Hamill et al. had reported on a syndromic phenotype of dilated cardiomyopathy with arrhythmias and ectodermal dysplasia in three unrelated young toddlers as early as 1988 (57). The children presented with episodes of recurrent VT by the age of 18 months, while chest pain and congestive heart failure developed by the 3rd year of life. Severe biventricular involvement with fibrosis and moderate inflammatory infiltrates was detected. Alopecia preceded the cardiac phenotype, while

TABLE 2 | Pediatric genetic testing overview.

	Who to test?	When to test?	How to test?	Why to test?
First test in family	- Youngest onset - Worst phenotype	- After complete cardiovascular evaluation - After constructing pedigree	- Full coverage of ARVC genes - Test that includes detection of copy number variants - Large panel a reasonable choice - With psychosocial support and multidisciplinary team	- To inform cascade screening of family. - Genotype-guided care
Cascade testing	- First degree relatives ≥ age 10, younger testing reasonable[a,b] - Families with a P/LP variant	- In conjunction with baseline cardiovascular screening	- Targeted sequencing of family variant(s) - With psychosocial support and genetic counseling	- Determine need for longitudinal cardiovascular screening - Inform decisions about exercise

References to substantiate statement: [a] = (51); [b] = (2).
P/LP, pathogenic or likely pathogenic variant as assessed by criteria published by the American College of Medical Genetics and Genomics and Association for Molecular Pathology (28).

lesions of PPK, nail and dental anomalies were noted despite their young age.

The homozygous pathogenic *JUP* variant is identified in other Aegean islands as well as in Turkey (58, 59). The prevalence of Naxos disease in Greek islands reaches 1:1,000 (60). Over the years, an increasing number of families with the phenotype of WH, PPK and ARVC/ACM left-dominant arrhythmogenic cardiomyopathy have been reported. Most of these reports concern pathogenic *DSP* variants (54, 61, 62). It is important to note that patients with Naxos or Carvajal disease are rare (outside of endemic regions), and that they present with a distinct cutaneous and cardiac phenotype. Regardless, many lessons were learned based on Naxos disease patients, as described below.

The Cutaneous Phenotype

In patients with Naxos disease, WH is apparent from birth. Interestingly, observant members of affected families have mentioned it as a harbinger of a severe heart disease (personal communication). In the largest reported series of pathogenic *DSP* variant carriers, WH was shown to have high specificity in detecting subjects who will develop a cardiomyopathy (62). In some patients with *JUP*/homozygous pathogenic variants (or biallelic), sparse WH or even alopecia were reported. Alopecia has been related mostly to *DSP* variants (63).

Keratotic lesions on the palms and soles generally develop during the second year of life when the child is using hands and feet (60, 63). In early infancy, eczematous lesions, fragile skin or even erosion and ulcers have been observed on the perioral and sacral areas or dorsal surfaces of the hands and legs (**Figure 6**). Nail dystrophy and dental anomalies may be part of the phenotype particularly of pathogenic *DSP* variants (61, 64). Pemphigus-like vesicular lesions on palms, soles and knees have been reported as well (65). Of note, neonatal lethal epidermolysis has been reported in both *JUP* and *DSP* homozygous variants (63, 66, 67).

Immunohistology of non-lesional skin on the forearm in pathogenic *DSP* variant carriers revealed that desmoplakin is irregularly localized in the basal layers of epidermis instead of the membranous distribution observed in normal skin. The same, albeit to a lesser extent, is observed for plakoglobin. In areas of clinically "normal" skin, reduced expression of connexin43 is also observed (62). Expression of desmosomal proteins in basal layers of epidermis are more representative of those expressed in myocardium.

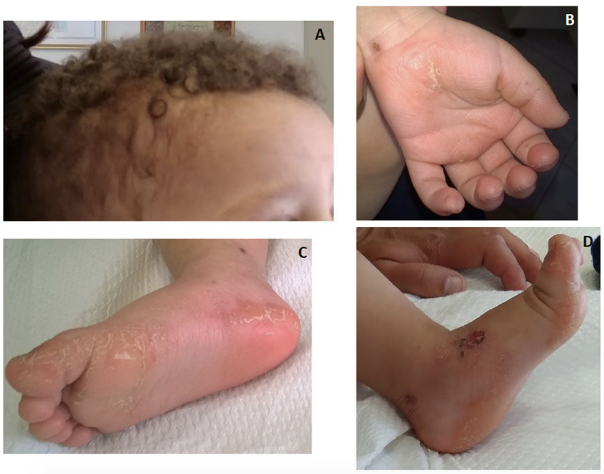

FIGURE 6 | An 18-month-old boy with Naxos disease, homozygous for a pathogenic *JUP* variant. Wooly hair apparent from birth **(A)** and mild hyperkeratosis on palmar and more prominent on plantar areas **(B)** and **(C).** Eczematous lesions and fragile skin with erosion on the dorsal foot area **(D)**.

The Cardiac Phenotype

While skin defects become apparent early in life, diagnostic features of ARVC do not develop until late puberty (54). The earliest observed cardiac abnormality was in a 5-year-old girl with the cutaneous phenotype of Naxos disease, homozygous for the *JUP* pathogenic variant. She presented with 14.000/24 h ventricular ectopics, without any detectable myocardial imaging change while, after a non-cardiac death, gross pathology of the whole heart and regular histology were normal. However, electron microscopy of the myocardium revealed biventricular gap junction remodeling and abnormal immunohistology for both connexin43 and plakoglobin at cell-cell junctions (68). Clinical presentation of ARVC as an episode of acute myocarditis has been highlighted also in a boy with Naxos disease. He had been followed-up since infancy due to

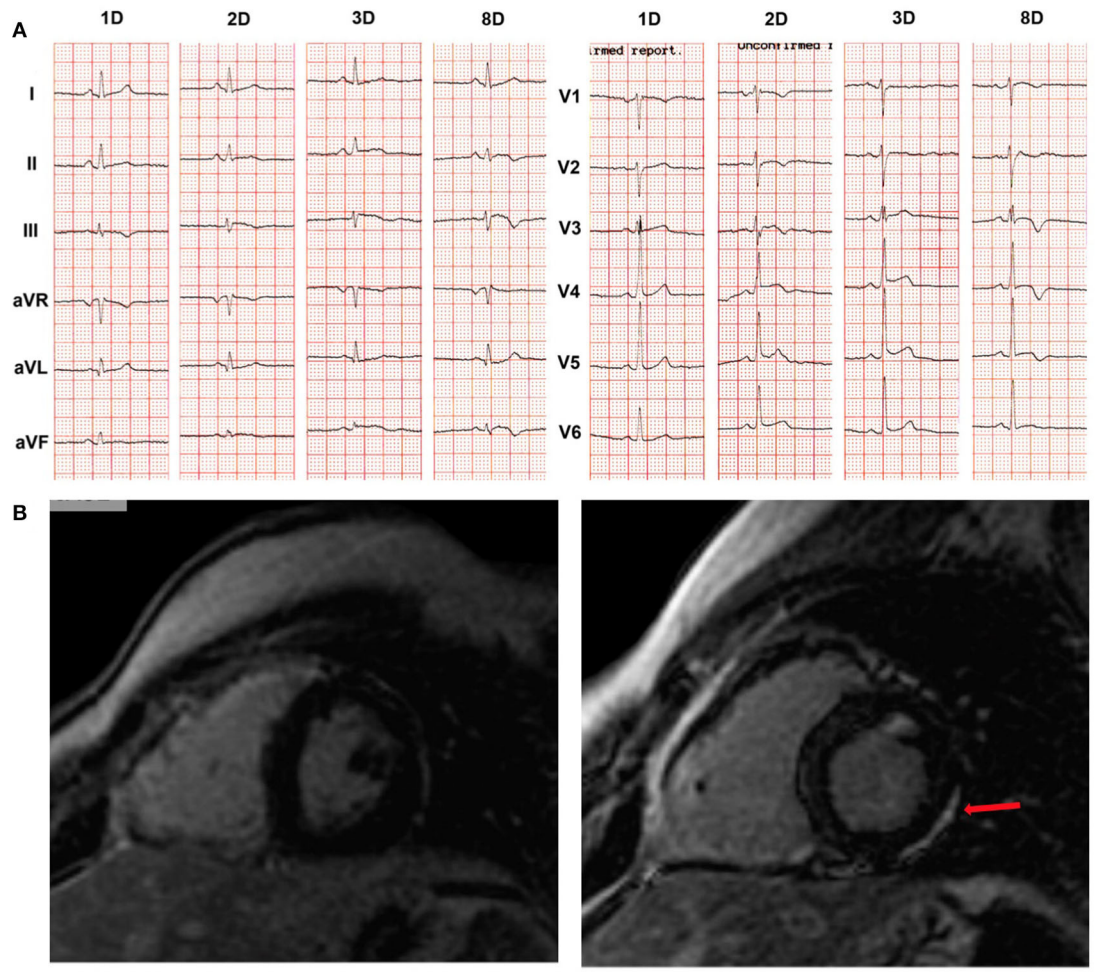

FIGURE 7 | Example of a boy with Naxos disease, homozygous for a pathogenic *JUP* variant, on regular follow-up since the age of 1 year old. He was asymptomatic until the age of 14 years when he was admitted with chest pain, increased troponin-I levels up to 20 μg/L (normal values: 0.04 μg/L), complex ventricular extrasystoles and changes on 12-lead ECG. **(A)** ECG recordings in 25 mm/s, 10 mm/mV, at the first (1D), second (2D), third (3D) and eighth (8D) days of hospitalization. Newly developed repolarization changes are observed. **(B)** Phase-sensitive inversion recovery CMR images to detect LGE 1 year prior to hospitalization (**left**) and the second day of hospitalization (**right**) are shown. No functional changes were observed on either CMR in both ventricles (i.e., normal LV and RV function). However, LGE was detected (arrow) in the LV myocardium **(right)** as compared to the isolated involvement of RV free wall, a year previously **(left)**, suggesting progression of disease. CMR, cardiac magnetic resonance; ECG, electrocardiogram; LGE, late gadolinium enhancement; LV, left ventricular; RV, right ventricular. Source: (69), obtained with permission.

the phenotype of WH and PPK, and he was a homozygous carrier of the pathogenic *JUP* variant (69). Newly developed patchy LGE changes on CMR and progression of repolarization changes were documented at the age of 14 years following an episode of chest pain (**Figure 7**). Therefore, a thorough cardiac investigation should be considered in any child with the phenotype of Naxos disease upon an episode of chest pain to detect potentially emerging myocardial defects or ARVC progression.

Symptoms of right heart failure in Naxos disease commonly appear later in the disease course. Isolated reports in toddlers homozygous or compound heterozygotes for *DSP* variants show that cardiomyopathy is clinically manifest with severe biventricular or predominant LV involvement leading to heart failure (60, 63, 70).

Cardiac biopsies performed in patients homozygous for a pathogenic *JUP* variant revealed fibrofatty replacement of RV myocardium. Inflammatory infiltrates were observed particularly when the biopsy was performed at the time of clinical progression (60). In a patient who fulfilled TFC for ARVC diagnosis and died suddenly at the age of 20 years, the RV showed extensive myocardial loss with fibrofatty replacement at subepicardial and medio-mural layers being regionally transmural with aneurysmal formations (68). While the LV appeared unaffected on gross pathology, areas of myocardial loss with fibrofatty substitution or replacement fibrosis with lymphocyte infiltrates were revealed

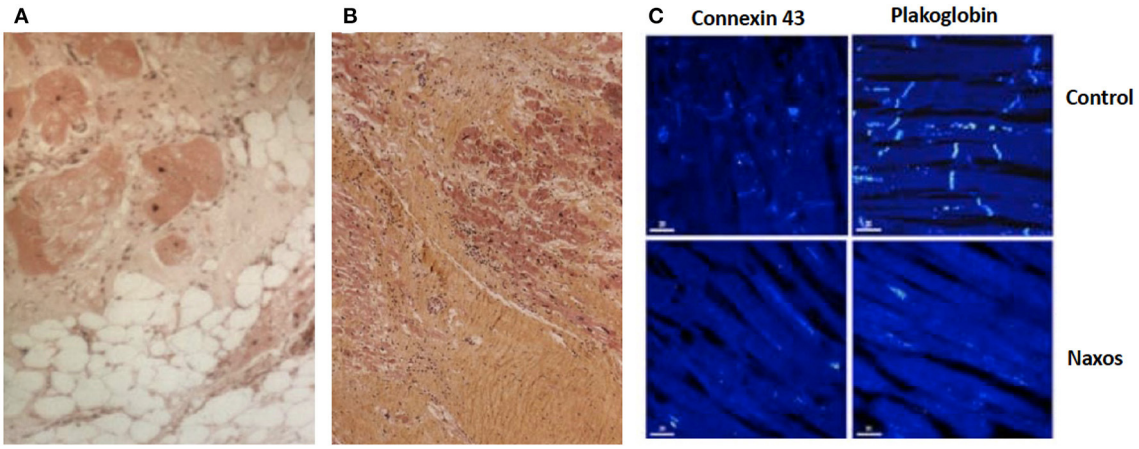

FIGURE 8 | Surgical sample from the anterior wall of the right ventricle of a 19-year-old patient with Naxos disease. Surviving myocardial fibers with some vacuolization, surrounded by fibrosis (haematoxyline-eosine-stained, magnification x 100) **(A).** Postmortem samples from the left ventricle of a patient with Naxos disease, who died suddenly at the age of 20 years. Myocardial loss with replacement fibrosis and lymphocyte infiltrates (haematoxylin-eosin-stained sections) **(B).** On confocal microscopy images (same patient) of the left ventricle, there is diminished signal of plakoglobin and connexin 43 at intercellular junctions **(C).**

on regular histology (**Figures 8A,B**). On immunohistology, the signal for plakoglobin and connexin43 were diminished at intercalated disks (**Figure 8C**).

The rare occurrence of the association of WH and PPK with ARVC illuminated the pathogenesis of the cardiomyopathy (71). Early detection of WH, skin fragility or PPK should warn for ARVC development later in life with variable penetrance. Therefore, a lifelong and targeted cardiac evaluation with 12-lead resting ECG, 24-h Holter monitoring and imaging not only with 2D-echocardiography but also with CMR and LGE is recommended.

Insights Into Disease Pathogenesis

Important insights into disease pathogenesis can be obtained from patients with Naxos disease (58). In a seminal study by Kaplan et al., electron microscopy of the myocardium revealed smaller and fewer gap junctions in both the RV and LV in a Naxos disease patients, which was accompanied by reduction of connexin43 expression at intercalated disks. Also, the amount of immunofluorescent signal for plakoglobin was significantly reduced at cell-cell junctions in both LV and RV tissue samples. As a result, an ultrastructural mechanism promoting activation delay and re-entry-based arrhythmia was suggested to be related to ARVC (68).

While this provides important clues on ARVC pathogenesis, this might not be the only disease mechanism in young ARVC patients since VT episodes have also been attributed to triggered activity (11, 72). In a recent study of patients diagnosed as CPVT, a *PKP2*-dependent electropathy was suggested as the pathogenetic mechanism of exercise-induced sudden death in those in whom postmortem examination did not reveal any myocardial structural abnormality (73). In several studies, it was shown that decreased desmosomal expression alters the properties of the sodium current and the velocity of the phase 0 upstroke of the action potential (74, 75). In *in vitro* and animal models, this causes significant activation delay, which increases propensity to functional block and promotes arrhythmia susceptibility at a very early disease stage (75, 76).

Also, a cardiomyocyte-specific *PKP2*-knockout mouse model showed that the loss of *PKP2* markedly reduced the transcriptional expression of genes required for intracellular calcium handling (77). Of note, in several published pediatric ARVC series, life-threatening ventricular arrhythmias have mostly been associated with structural abnormalities meeting the imaging TFC (11, 36, 78). Nonetheless, the true "electrical" onset of ARVC in children will be revealed only with prospective clinical cascade family screening.

Evolution of ARVC is a stepwise rather than a linearly progressive process (60). It comes through 'hot-phases' that may present as myocarditis-like episodes. A potential role of inflammation in ARVC was suggested as early as 1990 (79, 80) and has been increasingly reported since (69, 81–83). In these patients, the presenting symptom might be chest pain, palpitations or presyncope, followed by elevated cardiac troponin levels. Ventricular arrhythmias and repolarization changes on resting 12-lead ECG typically accompany the clinical picture (**Figure 7**). Abnormal CMR scans with early gadolinium enhancement suggestive of myocardial hyperemia and capillary leak, as well as T2 hyperintensity suggestive of myocardial edema may be detected. Generally, biventricular involvement is revealed.

The myocarditis-like "hot phases", either as the first clinical presentation or during disease evolution, suggest that an inflammatory process plays a role in disease progression and arrhythmogenesis. In a recent study, monozygotic twins presented with myocarditis at ages 17 and 18 (84). Extensive LV late gadolinium enhancement (LGE) indicative of fibrosis was observed on CMR, and a *DSP* variant was revealed in both

patients. In another study, features consistent with myocardial inflammation and severe ventricular arrhythmias were evident in six out of 32 pediatric patients (36). On a molecular level, ARVC is linked to local myocardial production of cytokines and alterations in the balance of circulating inflammatory cytokines.

NATURAL HISTORY

Three stages in ARVC evolution have been suggested: A "concealed" stage in which the disease is not evident on cardiac screening when only molecular changes can be detected; an "electrical" stage with abnormalities on ECG or Holter monitoring, but a normal or near-normal structure on conventional imaging; and a "structural" stage with expression of the full phenotype. Genotype-phenotype differences and the risk of life-threatening arrhythmias in the concealed stage remain controversial.

The symptomatic disease presentation in children has been reported around the 15th year of life, mostly in series with autosomal dominant ARVC (78). Frequent premature ventricular complexes (PVCs) and episodes of non-sustained VT have been observed as the first electrical manifestations in pediatric ARVC, whilst episodes of syncope or even cardiac arrest have been recorded in young individuals (11). In patients diagnosed under the age of 21 years, cardiac arrest was observed in probands only at a mean age of 15.3 years, and episodes of sustained VT at 16.7 years (11). Electrocardiographic and Holter abnormalities usually precede imaging changes of ventricular myocardium in children (85). Structural abnormalities in children with ARVC are often mild, and involve focal subtricuspid dyskinesia with preserved global function rather than severe RV enlargement (17). When biventricular involvement exists, ARVC is diagnosed in significantly younger age (12.4 $\pm$ 5.0 years) as compared to classic ARVC (16.7 $\pm$ 2.0 years) (36). Indeed, LV involvement has been related to worse prognosis in children with ARVC (58, 86).

CASCADE SCREENING

Given the strong familial disease pattern of ARVC, predictive genetic testing and/or cardiac evaluation should be performed in family members of ARVC probands to detect early signs of disease and prevent sudden cardiac death. Indeed, previous studies have shown that approximately one in three family members will develop ARVC (87–89) The yield of screening however varies due to the age-related and incomplete penetrance, and the ARVC disease spectrum can be diverse even among those carrying the same pathogenic variant (85). Ideally, cascade (genetic and/or cardiologic) screening is therefore performed within the confines of a multidisciplinary cardiovascular genetics program that is familiar with the complexity of this disease (2). The following sections will describe the evidence surrounding the questions of genetic as well as cardiac screening for ARVC.

Cascade Genetic Testing

First, a pedigree should be constructed before genetic testing in the family is started. If a family has multiple affected members, the individual with the youngest presentation and/or most severe disease should be tested first. This is recommended to maximize the chance that if there are multiple P/LP variants in the family they are all identified. As described earlier, a pediatric patient is particularly likely to have more than one P/LP variant.

Second, while a limited ARVC-specific panel is a reasonable choice for first-line genetic testing, increasingly large cardiomyopathy and arrhythmia next generation sequencing panels are used. A recent study has shown using these larger panels (i.e., next generation sequencing of cardiomyopathy and primary arrhythmia-associated genes) do increase detection of clinically informative P/LP variants although at the cost of increased detection of VUSs (90). Nonetheless, as children with ARVC may be particularly likely to have multiple variants, potentially in both desomosomal and non-desmosomal genes, using a larger panel is reasonable to maximize detection of the full genotype.

Third, a recent study by Van Lint et al. of 501 American, Dutch, and German ARVC probands found that P/LP ARVC-associated variants are almost never *de novo* (29). Therefore, if P/LP variant(s) are identified in a child, it is very likely a parent has transmitted the variant and so the parent and also any siblings are at risk. Anticipatory guidance for the family that this is a likely outcome is important.

Finally, children may also be offered genetic testing as part of cascade genetic screening. The age at which pre-symptomatic testing for ARVC is offered varies both within and between nations. Regardless, the decision can be complicated for some families, particularly as detection of a desmosomal variant will likely result in a discussion of the risks and benefits of exercise restriction. A decision-support tool for presymptomatic pediatric ARVC testing for has recently been developed and is publicly available (redcap.link/decision_aid; University of Alberta, accessed 6/20/21).

Cardiac Screening: "Who" and "When" to Screen for ARVC

Several research groups have shown that penetrance of ARVC is age-related: ARVC rarely manifests before the age of 10 years, and disease expression increases throughout life with the majority of pathogenic variant carriers expressing disease after 60 years of age (89). Some have suggested that this may be due to the developmental maturation of the intercalated disk, which completes at approximately 10 years after birth and subsequently requires time to get damaged by external and internal factors to ultimately elicit the ARVC phenotype (91). Consequently, most patients are diagnosed with definite ARVC between the second and fourth decade of life. Likewise, a recent study showed that the incidence of newly diagnosed ARVC follows an inverted U-shaped curve, which peaks in the 30–40 year age groups and is lowest at either end of the disease spectrum (**Figure 9**) (89).

Two recent studies using the same multicenter cohort of 502 definite ARVC patients described clinical features of patients with early (<18 years) or late (>50 years) ARVC presentation (11, 92). Overall, 15% presented as children or adolescents, of whom one in four presented with sudden cardiac death or resuscitated sudden cardiac arrest. Conversely, 21% of ARVC patients

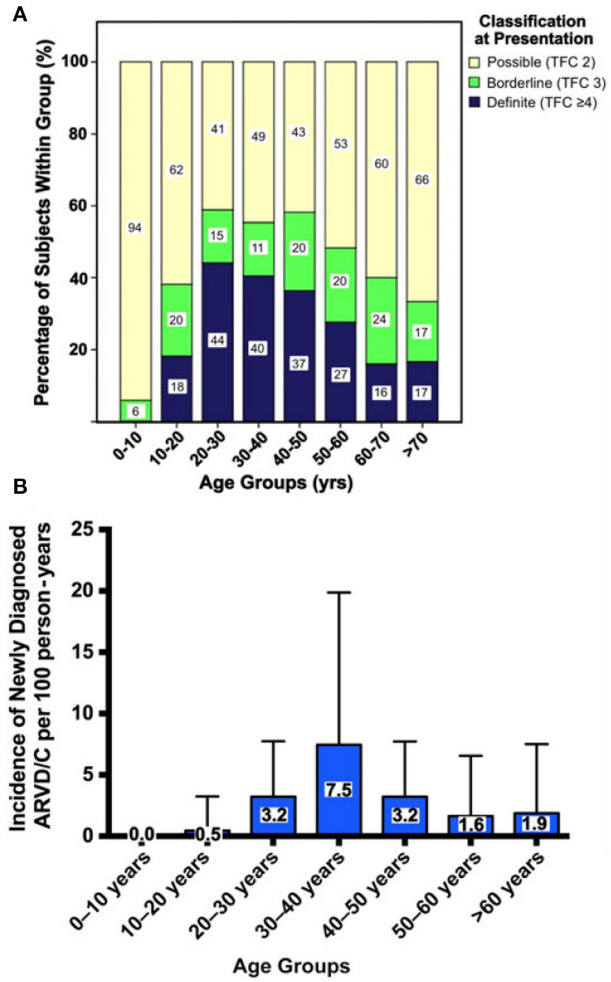

FIGURE 9 | Age-related penetrance of ARVC diagnoses among 274 family members screened for disease. **(A)** Cross-sectional prevalence of ARVC. All family members are stratified by age at evaluation, where each age group is scaled to 100% to show the proportion of family members with definite, borderline, or possible disease at time of evaluation. **(B)** Incidence of newly diagnosed ARVC per 100 person-years during prospective follow-up. Error bars denote upper limit of 95% confidence interval. Source: (89), obtained with permission.

presented after the age of 50 years, often with hemodynamically stable VT. While this suggests that all family members of ARVC patients are at risk of developing possibly lethal consequences of this disease, risk of ARVC among family members is not uniform. In a recent study of 274 first-degree family members of ARVC probands, predictors of ARVC diagnosis included symptoms, being a sibling to the proband, and presence of a pathogenic genetic variant (89). Recognition that symptomatic subjects have increased risk of ARVC is intuitive and should be a red flag in any clinical evaluation. The higher risk of disease in siblings as compared to parents and children may be considered surprising and was not completely explained by correcting for age in this study. This may suggest that other factors such as genetic background or shared environmental influences (e.g., exercise participation) impact disease development among family members. Consistent with a genetic inheritance pattern, pathogenic variant carriers have an increased risk of developing disease. Presence of a pathogenic genetic variant even conferred a 6-fold increased risk of disease in a cohort of 302 relatives from 93 families (87). These studies may help clinicians single out family members who should be more closely followed.

Cardiac Screening: "How to Screen" for ARVC

As described previously, ARVC is diagnosed by a combination of clinical tests as defined in the 2010 TFC. It should therefore be inferred that a full baseline evaluation including all TFC-prescribed tests is necessary to diagnose (or rule out) ARVC with certainty. However, many research groups have provided evidence that diagnostic yield differs among tests.

ARVC is a progressive disease (93), and therefore screening for disease development should be performed at multiple time points using the most useful combination of tests. Almost uniformly, studies have shown that 12-lead ECG and Holter monitoring are among the most sensitive tests for ARVC evaluation, with abnormal test results approaching 90% in affected patients (16). However, almost all these prior studies were cross-sectional in design, and did not consider progression that may have occurred on these tests. Te Riele et al. recently published a report on longitudinal follow-up in family members of ARVC probands (85). In their study, the authors followed 37 asymptomatic family members over a mean of 4.1 ± 2.3 years of follow-up. Overall, 11 (30%) subjects had evidence of disease progression (defined as the development of a new TFC at last follow-up, that was absent at enrolment) and 5 (14%) family members were diagnosed with definite ARVC at last follow-up. Disease progression was most often observed on 12-lead ECG (14%) or Holter monitor (11%), whereas progression on CMR (3%) was rare. In addition, "electrical" changes on ECG and Holter monitor preceded detectable "structural" changed on CMR, and the presence of both an abnormal "electrical" and "structural" substrate magnified arrhythmic risk (94). Taken together, these studies suggest that screening efforts may be most efficient by focusing on ECG and Holter monitoring, while imaging testing should be reserved for selected cases with symptoms and/or abnormal ECG or Holter monitoring tests. Of note, this does not substitute a full baseline evaluation and regular follow-up as per TFC.

Recommendations for Screening of Pediatric Family Members

In case of a pediatric family member, cascade screening is complicated by the legal and psychological issues that accompany medical interventions in a healthy minor. As such, the Heart Rhythm Society (HRS) Expert Consensus Statement recommends to start ARVC screening at an age when disease expression is expected, i.e., at 10–12 years (2). In contrast, the Heart Failure Society of America (HFSA) suggests that screening should start at an age of 6 years and should be intensified between the ages of 13–19 years (51). Both guidelines recommend the use

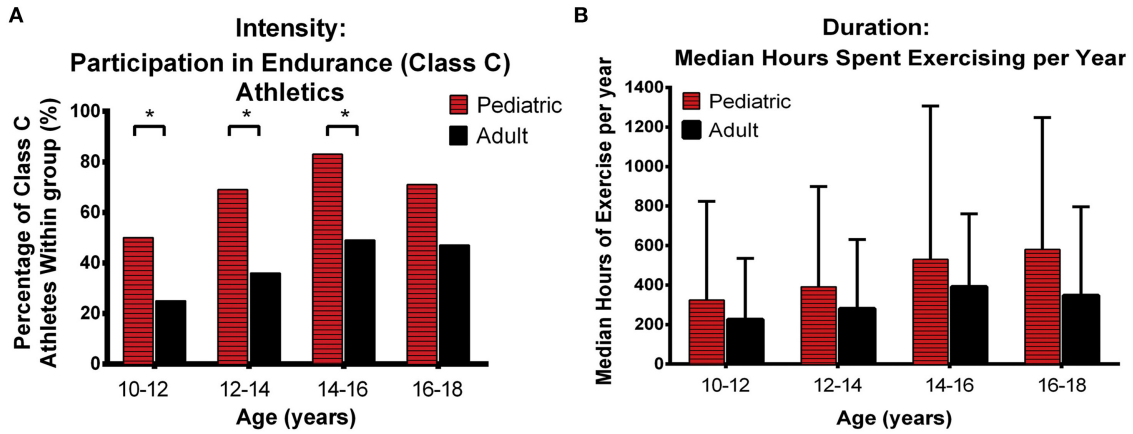

FIGURE 10 | Comparison of exercise intensity and duration during adolescence between patients with pediatric and adult-onset ARVC. Pediatric patients were engaged in higher intensity exercise **(A)** and performed more annual hours of exercise **(B)** during adolescence than arrhythmogenic right ventricular cardiomyopathy patients with Adult onset. Error bars indicate 95% confidence interval. * indicates statistically significant at $p < 0.05$. ARVD/C, arrhythmogenic right ventricular cardiomyopathy. Source: (11), obtained with permission.

of genetic testing as well as cardiac evaluation with an ECG, 24-h Holter monitor, and imaging (echocardiography or CMR). Given the peak of ARVC diagnoses in adolescents and young adults, the onset and interval of screening should be adjusted by age, and possibly several other factors including symptomatology and athletic activity.

MANAGEMENT OF ARVC

Lifestyle/Exercise

In ARVC patients, frequent high-intensity aerobic exercise has been associated with worse clinical outcomes in patients and increased penetrance in at-risk genotype positive relatives. Data from multiple ARVC cohorts consistently shows that competitive or frequent vigorous intensity endurance exercise is associated with earlier onset, sudden cardiac death presentation, worse survival free from ventricular arrhythmias in follow-up, worse RV and LV structure and function, and increased likelihood of heart failure and transplant (95–99). This pattern holds for both gene elusive patients (100) and those with a P/LP variant (95). Reducing exercise after diagnosis results in lower risk of VT and implantable cardioverter-defibrillator (ICD) therapy in follow-up (95, 101). Therefore, although wording varies, guidelines consistently recommend that patients with a definite ARVC diagnosis refrain from competitive or frequent vigorous/high intensity aerobic exercise. The evidence base for exercise guidance for ARVC patients and published clinical recommendations have been recently comprehensively summarized by Zorzi et al. (102).

Exercise has a similar negative impact on patients with the *TMEM43* p.S358L variant, with recent publications showing athletic individuals had earlier onset of ventricular arrhythmias and a considerably worse survival free from appropriate ICD-therapy in follow-up (103). In contrast, a recent publication of a cohort of patients and family members with *DSP* P/LP variants suggested history of exercise was less common in probands and that there was no association of exercise history with sustained ventricular arrhythmias or RV or LV dysfunction (48). While this study requires replication, particularly since it was not designed to measure the patient's exercise history, it does highlight the potential for genotype-specific exercise guidance in the future. Presently, it is important to recognize that current professional recommendations may not accurately reflect predominantly LV disease often associated with *DSP* and *PLN* variants as there is little data to draw on for these populations.

There has been limited information specifically focused on the impact of exercise in pediatric patients. Te Riele et al. analyzed exercise participation in 88 patients with P/LP variants (16 pediatric pediatric-onset, 72 adult-onset) who underwent detailed exercise interviews (11). As shown in **Figure 10**, pediatric patients were significantly more likely than those with adult-onset to have been involved in endurance (Class C) athletics before the age of 18 years (13 of 16 [81%] vs. 39 of 72 [54%]). Specifically, pediatric-onset ARVC was associated with endurance athletics in the 10- to 12-year ($p = 0.047$), 12- to 14-year ($p = 0.017$), and 14- to 16-year ($p = 0.026$) age categories (**Figure 10A**). In addition, patients with pediatric-onset disease participated in more annual hours of exercise during adolescence compared with patients with adult-onset ARVC, although this did not reach statistical significance (**Figure 10B**). Also of note, in a recent study of 101 family members of ARVC patients with P/LP variants (mostly in *PKP2*), Wang et al. showed that the difference in exercise between individuals who later developed ARVC and those who remained unaffected was higher in females than in males (104). Girls who had done high-dose exercise in adolescence had the worse survival free from diagnosis. This highlights the importance of "counseling adolescent (and adult) family members with a positive genetic test but who are phenotype negative that competitive or frequent high-intensity exercise is associated with increased likelihood of developing ARVC and ventricular arrhythmias" and engaging in shared decision-making regarding appropriate exercise participation

with the family as recommended by recent Heart Rhythm Society professional guidelines developed with multiple international cardiology and genetics societies (2).

Medical Management and Ablation

Medical therapy plays an important role in the management of patients with ARVC. It must be recognized that there are no prospective randomized or non-randomized clinical trials of any form of medical therapy in ARVC patients of any age. In this section, we will outline the approach to medical therapy employed by the Johns Hopkins ARVC program. It is our impression that this approach is similar to that employed at other high volume ARVC centers.

The cornerstone of medical therapy in ARVC is the use of beta blockers. We believe that the rationale for use of beta blockers is compelling. First, it is well established that beta blockers are the only form of medical therapy that has been shown to reduce the risk of a cardiac arrest in patients with various types of heart disease. Second, beta blockers have been proven to be of benefit in the treatment of patients with various forms of heart failure. Third, a great proportion of cardiac arrests that occur in ARVC patients occur during exercise (95), a situation where catecholamine levels are high. And fourth, Denis and colleagues have demonstrated that a high dose isoproterenol infusion (45 mcg/min) triggers long runs of polymorphic VT in ARVC patients and that this dramatic form of VT responds rapidly to administration of an intravenous beta blocker (105). As such, while conclusive evidence based on (non-)randomized trials is lacking, it is likely that beta-blockers (at least to some degree) prevent ventricular arrhythmias.

Angiotensin converting enzyme (ACE) inhibitors also play an important role in the management of ARVC patients with significant structural disease and RV and/or LV dysfunction. While there are no studies proving their value in ARVC patients, there are many trials demonstrating the critical role of ACE inhibitor therapy in patients with ischemic and non-ischemic forms of dilated cardiomyopathy. We have a low threshold to institute ACE therapy once a beta blocker has been started, assuming the patient tolerates it. Because this is an empiric approach and not proven, we stop the ACE inhibitor if the patient has side effects.

A third form of therapy that we do not employ but you should be aware of is the use of diuretics and nitrates to unload the RV. Fabritz and colleagues demonstrated the value of this approach in a mouse ARVC model (106). More recently, Kalantarian and colleagues published a retrospective analysis of six ARVC patients placed on load-reducing therapy with nitrates and diuretics. The authors reported less RV enlargement during a mean follow-up of 3 years (107). Despite these two studies showing a potential of benefit, we have not employed this form of therapy due to the likelihood of side effects and limitations in the design of these small trials. This is clearly an area for further investigation.

A final type of medical therapy commonly used in ARVC patients are antiarrhythmic drugs. Wichter and colleagues demonstrated the value of sotalol in ARVC patients decades ago (108). In contrast, a more recent study by Marcus showed little value of sotalol, and concluded that amiodarone had greater efficacy (109). Of note, there has been increased interest in the use of flecainide. Ermakov published a small series of combination antiarrhythmic therapy including flecainide (110). Moreover, Cerrone has shown in an animal model that flecainide is of value in a *PKP2* mouse model of ARVC (75). Based on these trials, a prospective randomized clinical trial of flecainide is under way, which is a short-term cross-over study where the endpoint is suppression of PVCs. At present, our preferred antiarrhythmic drug in ARVC patients is flecainide, and if not tolerated or effective we employ sotalol. While we have used amiodarone in the past, we much prefer proceeding to VT ablation rather than start a young person on amiodarone.

Indeed, radiofrequency ablation has gained popularity for ARVC management over the years. While initial studies have indicated that endocardial ablation procedures have a high VT recurrence rate in ARVC patients (111), much better results are obtained with a primary epicardial, or combined endo-epicardial approach (112, 113): in these studies, VT-free survival at 3 years follow-up may even be as high as 85% (114). It should be noted that these ablation results were obtained in high-volume tertiary care centers, and that these results may not pertain to centers that are unfamiliar with ARVC management and its predominantly epicardial substrate (115).

Arrhythmic Risk Stratification/ICD Implantation

Patients with ARVC have an average annual risk of approximately 10% to develop potentially life-threatening ventricular arrhythmias or sudden cardiac death (116). Of primary concern is sudden cardiac death prevention, for which the only effective treatment is the placement of an ICD. However, this treatment is invasive with inherent complication risk, and can impose physical or psychological burden to the patient. As such, estimating the probability of developing ventricular arrhythmias is pivotal to protect those at high risk, while at the same time limiting interventions among those who are unlikely to derive benefit from their results.

Over the years, many studies have evaluated risk factors for ventricular arrhythmias in ARVC. A full overview of risk factors goes beyond the scope of the present manuscript and can be found elsewhere (116). In short, established predictors of ventricular arrhythmia in ARVC include male sex, younger age at diagnosis, RV systolic dysfunction, prior non-sustained VT, syncope, and high PVC count on Holter monitoring.

Several expert consensus documents recently consolidated the available evidence on arrhythmic risk stratification in ARVC, including the 2015 international task force consensus statement on management of ARVC (117), the 2017 AHA/ACC/HRS guideline for management of ventricular arrhythmias (118), and the 2019 HRS consensus statement on evaluation, risk stratification and management of arrhythmogenic cardiomyopathy (2). While these publications presented a large step forward for clinicians taking care of ARVC patients, several limitations remained: first, these algorithms were based on expert opinion; second, all guidelines were flowchart-based and did not consider the potential interactive effects

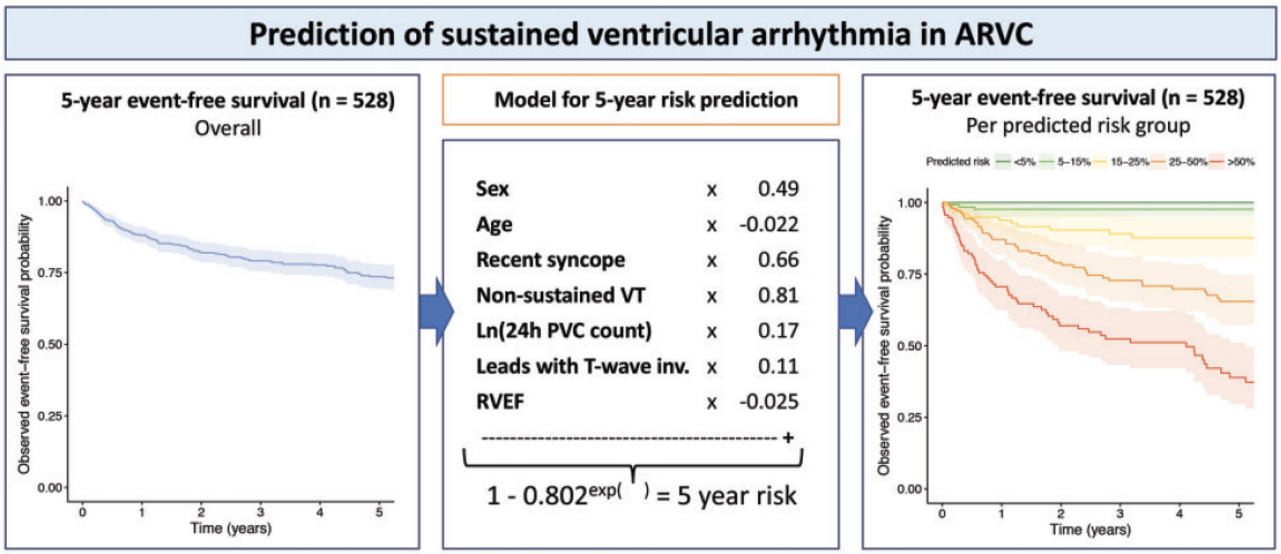

FIGURE 11 | Multivariable risk model to predict occurrence of first sustained ventricular arrhythmia in ARVC patients without prior arrhythmic events. Left panel denotes arrhythmia-free survival in 528 definite ARVC patients in North-America and Europe. Middle panel shows the derivation of a multivariable model for ventricular arrhythmia occurrence, using seven easily available clinical predictors. Right panel shows observed event-free survival stratified by predicted risk in the model. Source: (43), obtained with permission.

of combinations of risk factors; and third, translation to absolute risks was lacking. To address these shortcomings, a large transatlantic network of 15 centers in North-America and Europe recently published a multivariable model that enables quantitative individualized prediction of arrhythmic risk in ARVC (**Figure 11**), which is available for use at www.arvcrisk.com (43, 44). As of today, several studies validated the performance of the risk model in external cohorts, with good to excellent results (119–121). For the purpose of this review, it is however important to highlight that only a minority of study subjects were in the pediatric age range, and future studies should confirm the utility of the risk model in children and adolescents.

CONCLUSION

ARVC is an inherited heart disease characterized by fibrofatty infiltration predisposing the patient to ventricular arrhythmias and slowly progressive RV and LV dysfunction. The seminal description of Naxos disease led to the identification of genetic variants in the cardiac desmosome that are associated with ARVC. The disease is typically inherited in an autosomal dominant fashion, with incomplete penetrance and variable expressivity suggesting a strong role for environmental factors. Indeed, exercise exposure and myocardial inflammation have been linked to cardiac disease expression. Given that arrhythmias may occur early in the disease course, early screening and disease detection is of great importance. While there is no definitive cure for ARVC, treatment with beta blockers/antiarrhythmic medication, ACE inhibitors and load reducing therapy may reduce symptoms. Over recent years, several societies provided guidelines for ICD implantation. Future efforts should combine (inter-)national registries to improve our understanding of the clinical characteristics, genetic background, and prognostic factors associated with adverse outcome in ARVC patients at the pediatric age range.

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.

AUTHOR CONTRIBUTIONS

AR, CJ, HC, and AT conceived and designed the research and drafted the manuscript. AR and AT made critical revision of the manuscript for key intellectual content. All authors contributed to the article and approved the submitted version.

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Pediatric Restrictive Cardiomyopathies

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Restrictive cardiomyopathy (RCM) is the least frequent phenotype among pediatric heart muscle diseases, representing only 2.5–3% of all cardiomyopathies diagnosed during childhood. Pediatric RCM has a poor prognosis, high incidence of pulmonary hypertension (PH), thromboembolic events, and sudden death, is less amenable to medical or surgical treatment with high mortality rates. In this *scenario*, heart transplantation remains the only successful therapeutic option. Despite a shared hemodynamic profile, characterized by severe diastolic dysfunction and restrictive ventricular filling, with normal ventricle ejection fraction and wall thickness, RCM recognizes a broad etiological spectrum, consisting of genetic/familial and acquired causes, each of which has a distinct pathophysiology and natural course. Hence, the aim of this review is to cover the causes, clinical presentation, diagnostic evaluation, treatment, and prognosis of pediatric RCM.

Keywords: cardiomyopathy, restrictive cardiomyopathy (RCM), sarcomeric cardiomyopathy, pediatric cardiomyopathies, heart transplant (HTx)

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INTRODUCTION

Restrictive cardiomyopathy (RCM) is a heart muscle disease characterized by abnormal diastolic function with restrictive filling and normal ventricular size, wall thickness, and ejection fraction. Differently from hypertrophic, dilatated and right ventricle arrhythmogenic cardiomyopathy, where definition is based on morphology, RCM is defined based on physiology. However, under this common denominator, a wide spectrum of diseases are enclosed, with different causes, natural history, prognosis, and management. Furthermore, a restrictive hemodynamic profile can appear during the natural course of dilated and hypertrophic cardiomyopathy (HCM), being predictor of poor prognosis.

Among pediatric cardiomyopathies, RCM is the least common phenotype, representing only 2.5–3% of cardiomyopathies diagnosed during childhood (1). Unfortunately, compared to other pediatric cardiomyopathies, RCM is less amenable to medical or surgical treatment with higher mortality rates: 63% within 3 years of diagnosis and 75% within 6 years of diagnosis (2). As a consequence, rate for heart transplantation is relatively higher. Accordingly, within the Pediatric Heart Transplant Study Group, patients affected by RCM represents 12% of whole group of patients with cardiomyopathy undergoing heart transplantation.

Purpose of the present review is to summarize the causes of pediatric RCMs, their pathophysiology, clinical presentation and management.

DEFINITION AND EPIDEMIOLOGY

According to European Society of Cardiology position statement, RCM is defined as a myocardial disease characterized by impaired ventricular filling and normal/reduced diastolic volumes in the presence of (near) normal ejection fraction and myocardial thickness (3). Decreased active relaxation and increased parietal stiffness cause pressure within the ventricles to rise precipitously during diastolic filling, with only small increases in volumes. Although systolic function was classically said to be preserved in RCM, contractility is rarely normal, indeed. Furthermore, restrictive physiology can also occur in other scenarios, namely end stage HCM and dilated cardiomyopathy (DCM). However, it is suggested to consider these two entities apart. Restrictive cardiomyopathy is the least common among pediatric cardiomyopathies, accounting for only 2–5% of all cases, although the incidence may be higher in tropical areas of Africa, Asia, and South America, where endomyocardial fibrosis (EMF) is endemic (4). Its prognosis is poor with a 2-year survival <50%, being heart transplantation the only effective treatment (5, 6). Restrictive cardiomyopathy has been described in children of all ages, with mean age at diagnosis ranging from 6 to 11 years old in recent studies.

ETIOLOGY

Restrictive cardiomyopathies represent a heterogeneous group of cardiomyopathies which recognize several etiologies, including inherited and acquired causes. The term of idiopathic RCM is probably no longer appropriate in a large group of patients: in fact, genetics has identified mutations in various genes, above all sarcomeric genes. **Figure 1** summarizes the main causes of pediatric RCMs.

Idiopathic/Genetic RCMs

Although in the past decades pediatric RCMs were most commonly considered idiopathic in origin, the technical progress in genetics and the introduction and diffusion of next-generation sequencing technology into clinical practice have broadened the genetic spectrum of RCMs, discovering disease-causing genes among affected children. The Pediatric Cardiomyopathy Registry Investigators, through whole-exome sequencing of 36 genes involved in cardiomyopathies, reported pathogenic or likely pathogenic variants in 50% of children with RCM (7). Furthermore, not infrequently, they found patients with multiple candidate causal alleles, suggesting that the interaction effects from several alleles may be clinically relevant in pediatric cardiomyopathies.

RCM Caused by Sarcomeric Gene Mutation

Hereditary sarcomeric contractile protein disease finds expression in a broad spectrum of phenotypes. In pediatric RCM, sarcomeric mutations represent the most frequently identified genetic defect, accounting for one-third of children with idiopathic RCM (8). Particularly the genes reported are: myosin-binding protein (*MYBPC3*), β-myosin heavy chain (*MYH7*), myosin light chain genes, titin *(TTN*), troponin I (*TNNI3*), troponin T (*TNNT2*), and α-cardiac actin (*ACTC*) (8–13).

Although the primary molecular pathways dysregulated in RCM are poorly understood, some hypotheses have been advanced in last years based on experimental models. Sarcomeric gene defects may increase Ca^{2+} sensitivity for force development, impair the inhibitory properties of troponin, activate thin-filament-mediated sarcomeric contraction at submaximal calcium concentrations, resulting in increased muscle tension during diastole and in abnormalities of cardiac relaxation (14). A striking variability in the phenotype, age of onset, and disease severity, even within the same family with a definite sarcomeric mutation, is often documented (**Figure 2**) (15). The basis of this phenotypic plasticity is unknown: probably is multifactorial and not solely dependent from the consequence of the mutated protein on the sarcomere structure/function (16). This suggests that—differently from a pure Mendelian inheritance disorder—a group of modifier genes, each exerting a modest effect, together with epigenetic, post-transcriptional, and translational modifications of expressed protein and environmental factors are responsible for phenotype definition (15). Therefore, the clinical phenotype of a genetic disorder is not simply determined by the information contained in the causal deoxyribonucleic acid sequence: this has relevant consequences, not only for pathophysiological understanding of cardiomyopathies but also to unravel molecular pathways to propel molecular based treatment strategies.

RCM Caused by Cytoskeletal/Nuclear Gene Mutation

Desmin, lamin, and filamin C mutations share a wide heterogeneity in clinical presentation and, particularly, the possibility to determine a RCM, sometimes associated with skeletal muscle involvement and atrio-ventricular conduction disturbances early in the course of disease.

Desmin is a muscle-specific type III intermediate filament, important for the stability and correct cellular function, codified by *DES* gene (2q35). Notably, the spectrum of cardiac phenotypes associated with *DES* mutations ranges from dilated, arrhythmogenic, non-compaction, hypertrophic and, in rare cases, restrictive cardiomyopathies (17). Most of the known *DES* mutations are missense or small in-frame deletions. Many missense mutations introduce prolines that interfere with the hydrogen bonds within the peptide bonds of α-helices, thus destabilizing the protein structure. The majority of *DES* mutations are heterozygously inherited, indicating a dominant negative genetic mechanism or haploinsufficiency (18). However, a recessive autosomal transmission was reported in rare cases with compound heterozygous or homozygous *DES* truncating mutations (17). *DES*-related RCM may be associated with distal skeletal myopathy, atrio-ventricular blocks requiring pacemaker implantation and ventricular arrhythmias (19, 20). The ultrastructural characteristic is represented by granulofilamentous deposits in cardiac and skeletal muscle causing structural disorganization of the cytoskeleton leading to impairment of both myocyte relaxation and contraction (**Figure 3**).

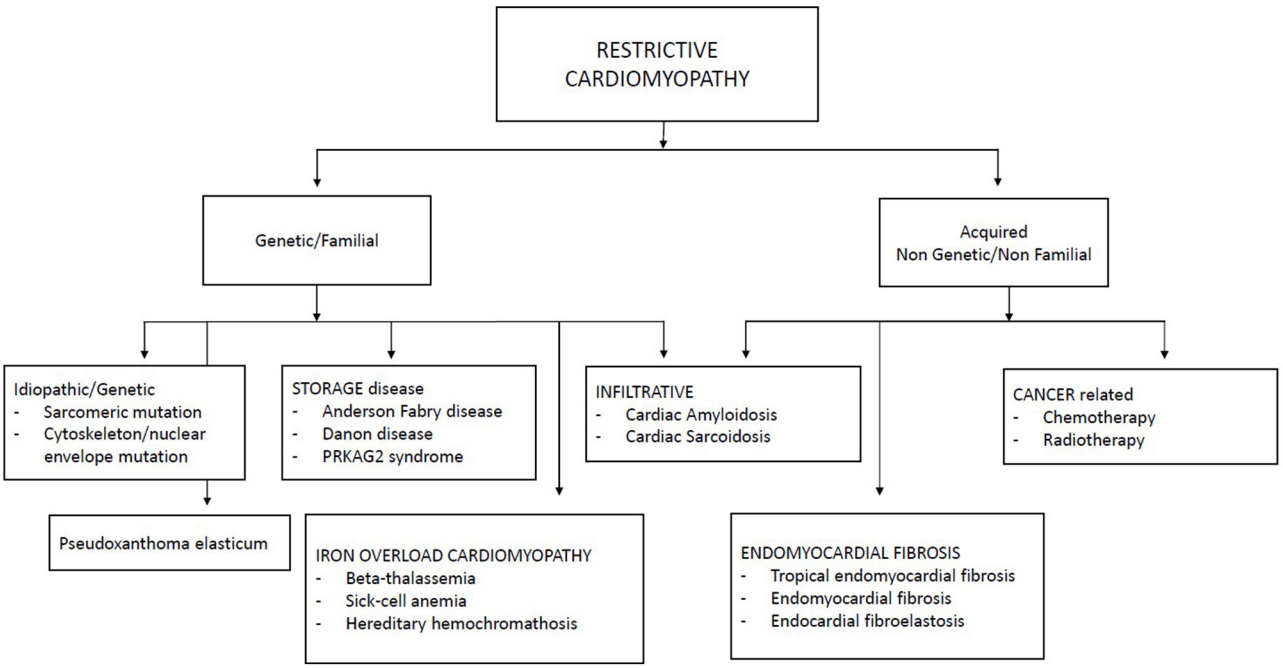

FIGURE 1 | Classification of restrictive cardiomyopathies.

Filamin C (or γ-filamin), coded by the *FLNC* gene (7q32.1), is member of a family of cross-link actin filaments expressed in cardiac and skeletal muscle, whose main role is to anchor membrane proteins to the cytoskeleton. Furthermore, filamin C is involved in protein degradation and autophagy control. The first association between RCM and *FLNC* mutations was described by Brodehl et al. in two unrelated Caucasian families with autosomal-dominant transmission, associated to atrial fibrillation and conduction disorders needing PM implantation (21). Cardiac histology showed myocytes hypertrophy with eosinophilic cytoplasmic aggregates due to mutated protein deposition, fibrosis, and mild disarray. Subsequent works by other groups reported families carrying *FLNC* missense mutations associated to variable degrees of a skeletal myofibrillar myopathy characterized by filamentous intracellular aggregates, combined with mild CK elevation, supraventricular arrhythmias, and RCM with early onset, often in childhood (22). Differently from missense mutation, *FLNC* truncating variants, were found in patients with a cardiac-restricted arrhythmogenic DCM phenotype characterized by a high risk of life-threatening ventricular arrhythmias (23).

Laminopathies are a heterogeneous group of diseases including heart disease, neuromuscular disorders, premature aging, and metabolic disorders, caused by mutation of *LMNA* gene (1q22), coding the nuclear envelope proteins lamin A and C, via alternate splicing. The spectrum of cardiac involvement ranges from supraventricular tachyarrhythmias and/or conduction system disease to DCM and ventricular tachyarrhythmias. Rarely *LMNA* can present as RCM in second decade of life, associated with atrio-ventricular blocks and requiring heart transplantation (24).

Storage Cardiomyopathies

Among lysosomal storage disorders, Anderson Fabry disease (AFD), Danon disease, and PRKAG2 are the most frequently associated with cardiac involvement, generally presenting as HCM.

Anderson Fabry disease is caused by a reduced or absent activity of alpha-galactosidase A due to mutations in the *GLA* gene, mapping on X-chromosome (Xq22). This results in progressive globotriaosylceramide accumulation, in different cytotypes and tissues, with consequent organs dysfunction. Overt heart involvement is rare in childhood and may determine ECG abnormalities and initial diastolic dysfunction (25).

Danon disease is an X-linked multisystemic disorder caused by a defect in the lysosome-associated membrane protein 2 (*LAMP2*) gene (Xq24), encoding the LAMP2 protein, leading to progressive accumulation of autophagic material. Clinical phenotype is characterized by heart and skeletal myopathy, cardiac conduction abnormalities, mild intellectual difficulties, and retinal disease. Men are typically affected earlier and more severely than women. Cardiomyopathy had classically a hypertrophic phenotype, with high risk of end stage evolution and need for heart transplantation at early age (26).

PRKAG2 syndrome is a rare, early-onset autosomal dominant inherited glycogen storage disease, due to *PRKAG2* gene mutation (7q36.1), coding for the c subunit of the AMP-activated protein kinase. It is characterized by ventricular pre-excitation, supraventricular arrhythmias, and cardiac hypertrophy. It is frequently accompanied by chronotropic incompetence and advanced heart blocks, leading to premature PM implantation (27, 28).

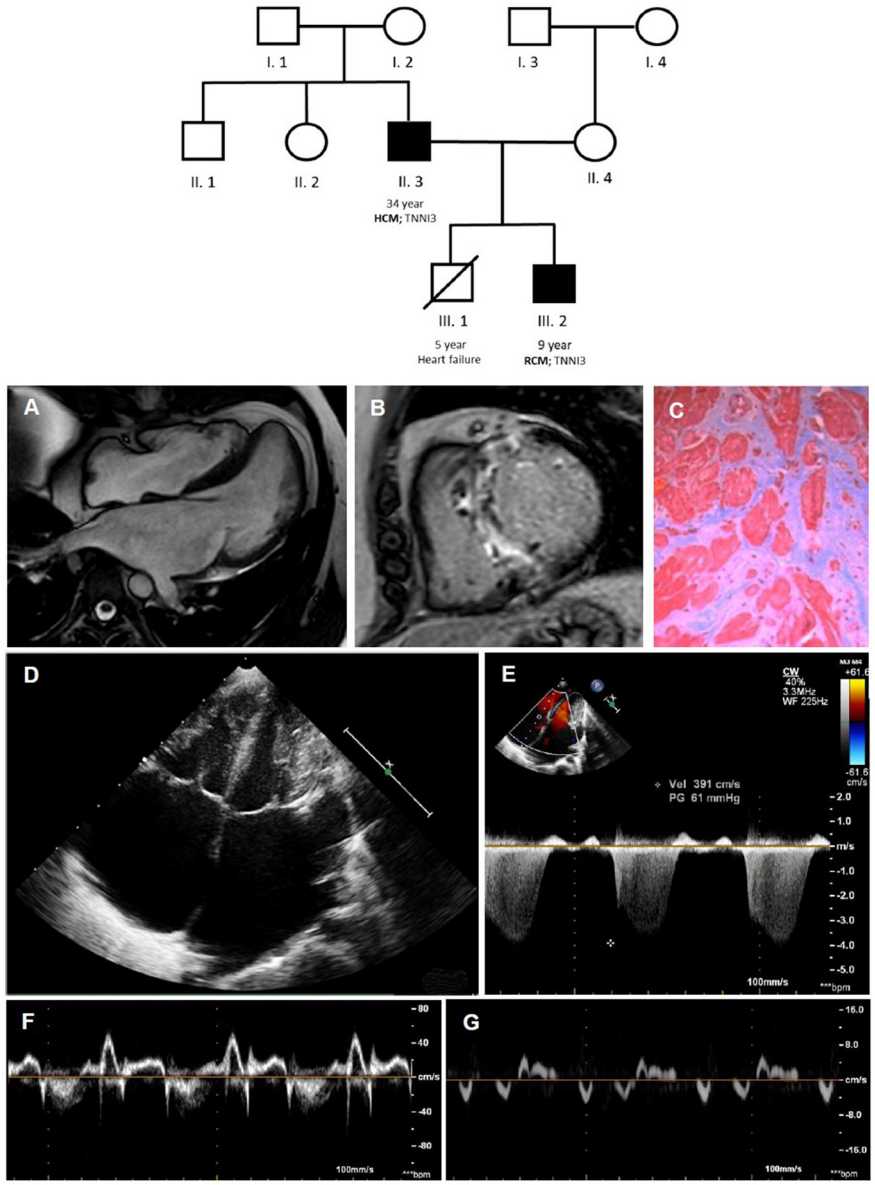

FIGURE 2 | Coexistence of HCM [father; II.3; **(A–C)**] and RCM [son; III.2; **(D–G)**] within the same family, due to a pathogenetic troponin I mutation. In the family tree, black filled symbols stand for affected carriers. Cardiac magnetic resonance shows mild septum hypertrophy with mild left atrial enlargement **(A)** and septal late gadolinium enhancement **(B)** and replacement fibrosis at optical microscopy **(C)**. The echocardiogram **(D–G)** shows a pediatric RCM with severe biatrial enlargement and small left ventricular volume **(D)** with signs of increased filling pressures at Doppler and TDI evaluation. HCM, hypertrophic cardiomyopathy; RCM, restrictive cardiomyopathy.

RCM Caused by Infiltrative Diseases

Amyloidosis is caused by deposition of an insoluble fibrillar protein called "amyloid" in the interstitium. It is extremely rare in childhood and is mostly seen late in life. Cardiac involvement is more commonly seen in transthyretin amyloidosis or light-chain amyloidosis, being mutated transthyretin amyloidosis (ATTRm) the earliest to occur in life, having been reported in the third decade of life (1).

Sarcoidosis is a multisystem, granulomatous disease of unknown etiology, characterized by non-caseating granulomas. Pediatric sarcoidosis is an extremely rare disease, with an estimated incidence of 0.6–1.02/100,000 children and a mean age at diagnosis of 11–13 years (29). Pediatric cardiac sarcoidosis is even rarer, though anecdotal cases were reported (30).

Iron Overload Cardiomyopathy

Iron overload cardiomyopathy (IOC) results from iron accumulation as a consequence of excessive iron intake or absorption. Increased iron intake is generally caused by multiple red blood cell transfusions for chronic anemia (e.g., thalassemia,

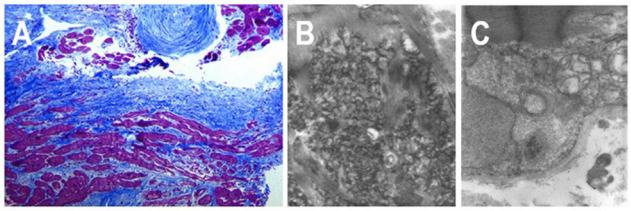

FIGURE 3 | Endomyocardial biopsy from a 18 years-old patient affected by a desmin-related restrictive cardiomyopathy, carrying a pace-maker for complete atrio-ventricular block. **(A)** Histologic section shows severe interstitial fibrosis and morphological myocytes' abnormalities with cellular hypertrophy and cytoplasmic vacuolization. **(B,C)** Transmission electron micrographs show multiple deposits of granulofilamentous material in the intermyofibrillar region.

sickle cell disease, hemolytic anemias, inherited bone marrow failure syndrome, myelodysplastic syndrome) or less commonly by infusions of iron-containing products used to treat certain porphyrias. Increased iron absorption is mainly caused by hereditary hemochromatosis (HH), due to mutations in genes involved in iron metabolism, increasing gastro-intestinal absorption. Among the known four HH subtypes, type 2 (also called juvenile hemochromatosis) typically presents by the second decade with a more severe phenotype accompanied, in addition to cardiomyopathy, by hypogonadotropic hypogonadism, arthropathy, and liver fibrosis or cirrhosis. Type 2 HH can be caused by two different genes: *HFE2* gene mutation, encoding hemojuvelin, a protein that interacts with hepcidin (subtype 1A) or *HAMP* gene, codying for hepcidin, a key regulator of circulating iron (subtype 2B). Regardless of its origin, IOC is characterized by a RCM with prominent early diastolic dysfunction which progressively evolute to an end-stage DCM (31). Although immunoinflammatory and inherited component may contribute to cardiac injury, iron overload plays a central role in the pathophysiology of IOC. Iron toxicity has been attributed to the production of free oxygen radicals, as a result of free iron availability. Excessive circulating free iron (i.e., not bound to transferrin) enters the cardiomyocytes, mainly through voltage-dependent L-type Ca^{2+} channels, in the form of Fe^{2+} (ferrous iron). Inside the cardiomyocytes, free iron induces the formation of reactive oxygen species, hence causing peroxidative damage of cellular structures (lipids, proteins, nucleic acids), cellular apoptosis, and finally cardiac dysfunction (32). Furthermore, iron overload increases calcium influx, which might impair, in its turn, diastolic function. A post-mortem hearts series (comprising two children) from patients with severe cardiac siderosis and heart failure leading to death or heart transplantation, except the severe granular iron deposition on Perl's stain, did not reveal replacement fibrosis and minor interstitial fibrosis was also unusual and very limited in extent, underlining the potential reversibility of heart failure in IOC (33). The best validated method for quantifying myocardial iron overload *in vivo* is T2* mapping with CMR. A T2* value of <20 ms at 1.5 T, typically measured in the interventricular septum, is used as a conservative cut-off for segmental and global heart iron overload and patients with the lowest T2* values have the highest risk of developing arrhythmia and heart failure (34).

RCM Caused by Fibrotic Process

- Tropical EMF is the most common cause of RCM, affecting more than 12 million people worldwide. Initially described in Uganda, EMF is commonly reported in rural populations of equatorial developing countries, where exhibits a bimodal distribution, peaking at 10 and 30 years of age. Many etiological hypotheses, not mutually exclusive, have been proposed: malnutrition, parasitic infections, environmental factors, and genetics. Due to regional differences in disease prevalence, a geochemical basis has been advanced as unifying hypothesis (35). Despite different possible candidates (magnesium deficiency, cerium toxicity, cyanogenic glycosides, high vitamin D, serotonin toxicity, herbal preparations), for none of them definitive evidence is available. The natural history of EMF is characterized by recurrent hot phases with inflammation and eosinophilia, progressing to a chronic phase where a biventricular RCM, caused by deposition of fibrous tissue in the endomyocardium, affects both ventricles or less frequently exclusively the right ventricle. In this last case, chronic venous hypertension causes facial edema and exophthalmos, jugular venous distention, hepatomegaly, and ascites, often out of proportion to peripheral edema (36).
- Eosinophilic EMF is a rare cause of RCM, resulting from toxicity of eosinophils toward cardiac tissues in patients with a hypereosinophilic syndrome (HES). Although the causes for eosinophilic infiltration of myocardium are various (hypersensitivity, parasitic infestation, systemic diseases, myeloproliferative syndrome, and idiopathic HES), pediatric HES is commonly associated with chromosomal abnormalities, and in 40% of the cases, it has been associated with acute leukemia (37). Eosinophil-mediated heart damage evolves through three stages, although these stages may be overlapping and not clearly sequential. The *acute necrotic stage* is characterized by infiltration of eosinophils and release of their granules' contents in the myocardium (eosinophilic myocarditis). Thereafter, an *intermediate phase* follows, with thrombus formation along the damaged endocardium (more often in the apex of the left ventricle) and finally a *fibrotic stage* characterized by reduced ventricular compliance and RMC. At this stage, entrapment of the chordae tendineae can lead to mitral and tricuspid regurgitation (38).
- Endocardial fibroelastosis is a congenital disease characterized by diffuse thickening of the LV endocardium secondary to proliferation of fibrous and elastic tissue, leading to early death. It manifests with a DCM phenotype or with a RCM phenotype with a small LV cavity. Most forms of endocardial fibroelastosis are associated with congenital heart diseases, first of all hypoplastic left heart syndrome and aortic stenosis/atresia, but also coarctation of the aorta, patent ductus arteriosus and, mitral regurgitation. In the minority of cases, a familial recurrence is seen, with all possible pattern of inheritance reported (39). Despite various attempts to unravel its origin, a definite mechanism could

not be identified. Genetic predisposition, viral infection (particularly mumps virus), and hypoxia during fetal cardiac development have been proposed as putative causes. Cardiac transplantation is required for end-stage heart failure (39). A promising surgical approach to remove endomyocardial layer showed improvement in the restrictive physiology together with growth of the left ventricle in parallel with somatic growth (40).

- Pseudoxanthoma elasticum is an inherited systemic disease of connective tissue, affecting skin, retina, and cardiovascular system. It is transmitted in an autosomal recessive manner and is caused by mutations in the *ABCC6* (ATP binding cassette subtype C number 6) gene (41). Histology of affected tissues exhibits elastic fiber mineralisation and fragmentation (so called "elastorrhexia") (42). Restrictive cardiomyopathy in relation to diffuse endocardial fibroelastosis is very rare (43).

Oncological Cardiomyopathy

Progress in cancer therapeutics over the past years has significantly improved survival rates for most childhood malignancies. Unfortunately, the developing cardiovascular system of children and adolescents is particularly vulnerable to most pediatric cancer protocols, relying on cytotoxic chemotherapy and radiation. Indeed, cardiac-specific disease is the most common non-cancer cause of death among long-term childhood cancer survivors, only second to the recurrence of primary cancer and the development of second cancers (44).

Anthracyclines (such as doxorubicin, daunorubicin, and epirubicin), used to treat hematologic cancers and solid tumors, are among the most used chemotherapeutic agents causing cardiotoxicity. Although the typical manifestation of cancer drug induced cardiomyopathy is a DCM, with LV dilation and thinning of myocardial wall, with "restrictive" physiology in the more advanced stages, a not negligible proportion of long-term survivors will eventually develop a RCM. Importantly, patients may present cardiotoxicity many years after treatment completion, needing carefully monitoring for years by echocardiography. However, despite the adverse cardiac effects of anthracyclines, these drugs are fundamental components and standard of care for many types of cancer. Risk factors identified for cardiotoxicity include: female sex, younger age at diagnosis, black race, trisomy 21, and certain lifestyle behaviors (1). Although a total cumulative anthracycline dose >300 mg/m^2 was identified as significant risk factor for late-occurring anthracycline-induced cardiotoxicity, adverse effects were reported also with lower cumulative doses (45).

Radiotherapy is frequently used together with surgery/chemotherapy in thoracic malignancies and lymphomas. Cardiac exposure is generally due to "stray" radiation as the heart is almost never the actual target, except for rare sarcomas or metastases (46). Although modern planning and irradiation techniques have significantly improved, radiation induced cardiac injury represents an actual issue, and combination with chemotherapy and novel agents increase cardiac toxicity. Restrictive cardiomyopathy is the consequence of a diffuse biventricular fibrosis, most often with a non-ischemic pattern, which reduces myocardial compliance. However, coexistent radiation induced micro and macrovascular disease can result in ischemia/infarction and regional fibrosis.

In the next years the number of the long-term cancer survivors is expected to rise, not only for improved long-term survival rates but—unfortunately—also for the increased incidence of many histological subtypes of childhood cancer: consequently, amelioration of prevention and treatment strategies is needed.

EMODINAMICS

Restrictive cardiomyopathy recognizes a unique hemodynamic profile, independently from the specific cause at the basis of diastolic dysfunction. In RCM impairment of diastole can be related both to the abnormal myocardial relaxation (i.e., the active actin-myosin cross-bridge detachment) and to the increased myocardial stiffness due to the myocardial cells (e.g., titin) and the interstitial matrix (fibrosis) alterations, determining elevated left and right-sided filling pressure. Although left ventricular ejection fraction is typically preserved, systolic contractility is often impaired as showed by tissue Doppler imaging and speckle tracking. Systo-diastolic dysfunction leads to reduced stroke volume. In the protodiastole—despite delayed active relaxation—there is an unusually early rapid filling of the ventricles, due to high atrial pressures, halted by incompliant ventricular walls from the end of the first third of diastole onward—reflecting myocardial stiffness. This results in a prominent "y" descent on the atrial pressure curves and, sometimes, in the *square root* or *dip and plateau sign* on ventricular pressure curves, consisting in an early decrease in ventricular diastolic pressure followed by a rapid rise to a plateau phase. During the following atrial contraction, the stiff ventricles are unable to easily accept additional blood volume, and thus the contribution from atrial contraction is often minimal. Differently from constrictive pericarditis (CP), in patients with RCM there is not enhanced ventricular interdependence, with concordant left and right ventricular pressures during the respiratory cycle and parallel changes in their pressure curve areas. Moreover, atria progressively enlarge, due to high intracavitary pressures and thin and distensible walls, predisposing to atrial arrhythmias and thromboembolic episodes. A relevant proportion of patients develop pulmonary hypertension (PH) with elevated pulmonary vascular resistances, unresponsive to vasodilator testing, precluding them form orthotopic heart transplantation (47).

CLINICAL PRESENTATION

The clinical presentation of RCM can be highly variable in children population, ranging from asymptomatic to right and/or left heart failure with PH.

Biventricular systolic function is typically normal until advanced stages of the disease, leading to heart failure with preserved ejection fraction (HFpEF). Consequently, clinical presentation is characterized by dyspnoea, poor appetite, ascites, peripheral edema, and hepatomegaly. However, while in adults with RCM symptoms and signs of heart failure are easy to detect, clinical evaluation in children is challenging because of the non-specific findings, resulting in some delay

in correct diagnosis. Besides, children with RCM may have a history of frequent respiratory infections. Progressive atrial enlargement can also lead to atrial arrhythmias such as atrial fibrillation and thromboembolic complications, with mitral and tricuspid functional regurgitation frequently associated, due to anulus dilatation. Wolff Parkinson-White syndrome with supraventricular tachycardia has also been reported.

Sudden death occurs in ≈25% of pediatric RCM patients, with an annual mortality rate reported of 7%, being cardiac ischemia, arrhythmias, and thromboembolic events the main responsible. Various risk factors for sudden death in pediatric RCM have been identified in previous studies: cardiomegaly, thromboembolism, raised pulmonary vascular resistance, pulmonary venous congestion, syncope, chest pain, left atrial size, PR and QRS duration. Albeit inconsistently sometimes, the main limitation of these studies is their retrospective nature and the intrinsic bias associated. Rivenes et al. evaluated a cohort of 18 pediatric patients with RCM who had sudden, unanticipated cardiac arrests, identifying chest pain, and syncope as risk factors for sudden death (48). Histopathologic evidence for ischemia was found in the majority of patients who died and the evidence of ischemia at Holter monitoring (i.e., ST depression) predicted death within several months. They proposed lethal ventricular arrhythmias as cause of death, showing examples of ventricular tachycardia/fibrillation recorded during resuscitation attempts (48). Complementary, Walsh et al. in a 16 pediatric RCMs cohort reported five sudden cardiac events, with three patients having complete heart block. In one of them, ST-segment elevation was documented before the onset of complete heart block, suggesting an underlying ischemic process as trigger of the bradyarrhythmia. Older age at presentation, longer PR interval and QRS duration were associated with sudden cardiac events (49).

DIAGNOSIS

Approximately 98% of RCM patients have an abnormal electrocardiogram (ECG) (50). The most common abnormalities are right and/or left atrial enlargement (91% of patients) (**Figure 4**). ST-T segment and T waves abnormalities are also frequently present and may be most evident at higher heart rates. In a small cohort of 12 children affected by RCM, Hayashi et al. found that obliquely elevated ST-T segments and notched or biphasic T waves were the most frequent ventricular repolarization abnormalities (67% of patients). Besides, the criteria for biventricular hypertrophy based on QRS voltage were achieved in seven patients (although three of them actually had some degree of hypertrophy at echocardiogram) (51). ST-T depression is usually mild and non-specific, but in some cases can be pronounced, mimicking a left main or proximal left anterior descending artery occlusion or a multivessel coronary disease. This was associated with high risk of sudden cardiac death (48) and Selvaganesh et al. hypothesized that marked ST depression can be caused by high end-diastolic pressure, impairing perfusion in the subendocardial region or stretching the myocardium and activating stretch sensitive channels (52). Conduction abnormalities can also be seen, as well as right or left ventricular hypertrophy signs (48). Furthermore, serial ECG-Holter monitoring is useful for the evaluation of rhythm disturbances and ST segment analysis.

Chest X-ray is abnormal in nearly 90% of cases and usually show cardiomegaly and pulmonary venous congestion (51).

Echocardiography plays a key role in RCM diagnosis. Marked biatrial enlargement with a normal or slightly decreased LV ejection fraction are widely considered as pathognomonic findings (**Figure 5**). Regardless of etiology, the considerable elevation in filling pressures in patients with RCM is reflected in abnormal mitral inflow and tissue Doppler variables. A short (<140 ms) mitral deceleration time, increased pulsed wave (PW) Doppler mitral E/A ratio (>2.5) and E/e′ >15 are markers of significantly elevated left filling pressures (53). Although IVRT (isovolumic relaxation time) is prolonged when myocardial relaxation is impaired, due to delayed LV pressure falling during the isovolumic relaxation, a short LV IVRT of <50 ms is frequently detected in RCM, as consequence of the high LA pressure (53). Typically plethoric inferior vena cava and hepatic veins are seen and, with inspiration, diastolic flow reversal in the hepatic veins is documented due to the inability of a non-compliant right ventricle to accommodate the increased venous return. Echocardiography is helpful in differential diagnosis between RCM and CP. Although both conditions shares E-wave predominance and short deceleration time, respirophasic shifting of the interventricular septum, caused by exaggerated interventricular dependence, is characteristic of CP. Accordingly respiratory flow variations consisting in increasing >25% in mitral inflow during expiration and >40% in tricuspid inflow after inspiration are absent in RCM but frequently noted in CP (54). However, among all echocardiographic parameters, the most useful to distinguish RCM from CP are those of tissue Doppler imaging. In fact a normal tissue Doppler e′ velocity (>8 cm/s) indicating normal LV relaxation virtually excludes RCM. In patients affected by CP, where diastolic dysfunction is due to pericardial constraint, e′ is normal or even increased since the longitudinal movement of the myocardium is enhanced because of constricted radial motion. Furthermore, in patients with pericardial constriction, lateral mitral annular e′ is usually lower than e′ from the medial annulus (so called "*annulus reversus*") (55). This finding, absent in RCM, reflects the tethering of the lateral mitral annulus to the adjacent fibrotic and scarred pericardium. In this regard, speckle tracking may add even higher diagnostic accuracy in differentiating constriction from restriction: in fact while in RCM both radial and longitudinal strains are reduced due to a diseased myocardium, in CP reduction mainly involve circumferential strain, reflecting the subepicardial tethering offered by the fibrous pericardium (55, 56). The myocardial performance index (MPI) or Tei index may provide further information of both LV diastole and systole, with normal value of 0.33 ± 0.02 from 3 to 18 years old. It is defined as the sum of the isovolumic contraction and relaxation times divided by the ejection time, and it can be calculated from PW Doppler at the mitral and aortic valve simultaneously or using TDI at mitral valve annulus (57).

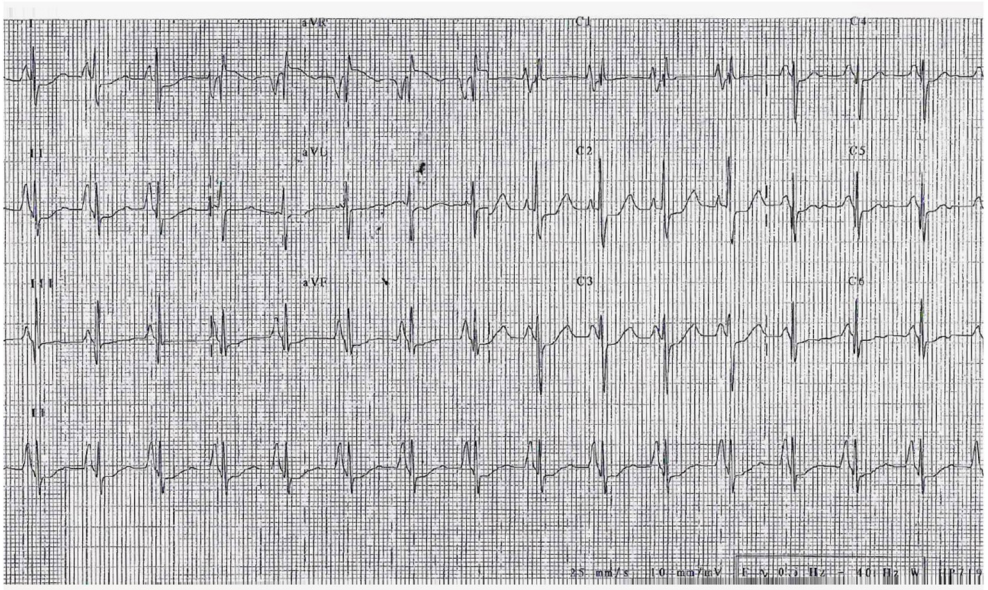

FIGURE 4 | ECG of a 3-year-old girl, affected by restrictive cardiomyopathy, showing biatrial enlargement and diffuse repolarization abnormalities. In the peripheral leads, please note the *monstre* atrial enlargement.

Moreover, lateral a′ velocity (cut-off ≤0.042 m/s) and pulmonary vein A wave duration (cut-off ≥156 m/s) both have sensitivity and specificity ≥80% for LVEDP ≥ 20 mmHg measured on right heart catheterization (58). Ancillary methods such as pulmonary regurgitant flow velocity, color M-mode flow propagation, and myocardial velocity gradient have been proposed for differential diagnosis between RCM and CP, however data in pediatric age are lacking. Finally, the performance and interpretation of diastolic measurements in children are challenging, given the higher heart rates, potential need for sedation, together with conflicting and limited data on the relationships among the diastolic variables and the degree of dysfunction (1). Indeed, in the assessment of diastolic dysfunction among 175 children with cardiomyopathy, the percentage of normal diastolic variables in children with overt cardiac dysfunction was high, with discordance between e′ and left atrium (LA) volume criteria. Patients with RCM were best identified with mitral E deceleration time, which was found to be abnormal in 75% of patients (59). In another study, Sasaki et al. described the LA area indexed to body surface area as the most useful measurement to differentiate between healthy children and RCM patients (60).

Cardiac magnetic resonance (CMR) offers a better spatial resolution than echocardiography, providing detailed information about anatomic structures, ventricular function, perfusion, and tissue characterization (61). For instance, T2*-weighted CMR is the diagnostic gold standard to detect and quantify myocardial iron content in IOC (62). Late gadolinium enhancement (LGE) can show peculiar patterns of replacement and reactive fibrosis, which can direct the diagnosis to specific subtypes of RCM. In AFD, LGE is typically localized in the infero-lateral mid-basal wall of left ventricle, and because of the distinctive fatty nature of the intracellular deposits, native T1 mapping has typically a low value, in contrast to most of other infiltrative or storage cardiomyopathies (63). In cardiac sarcoidosis LGE distribution is patchy, often with multifocal distribution, not following a coronary artery topography, sparing the endomyocardial layer, and involving mainly the basal and lateral LV walls (64). When performing CMR, it must be considered that the young patients must hold still in the scanner and follow the instructions to minimize motion artifacts during image acquisition. Whereas, this is possible in older children (more than 6–8 years of age), it requires sedation and anesthesia for younger patients, with different possible strategies, often depending on institutional preference and availability of resources such as pediatric anesthesiologists (65).

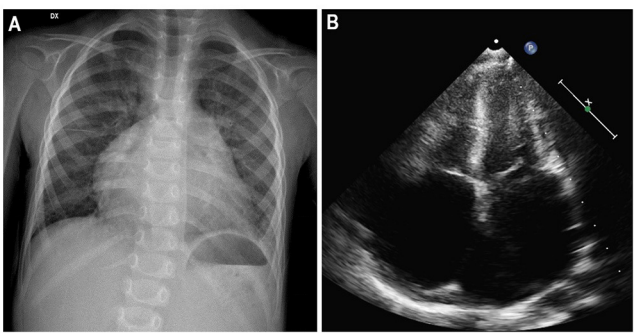

FIGURE 5 | Three-year-old girl affected by restrictive cardiomyopathy. Antero-posterior chest radiograph **(A)** showing massive cardiomegaly due to severe biatrial enlargement, as confirmed by apical four-chamber echocardiography **(B)**.

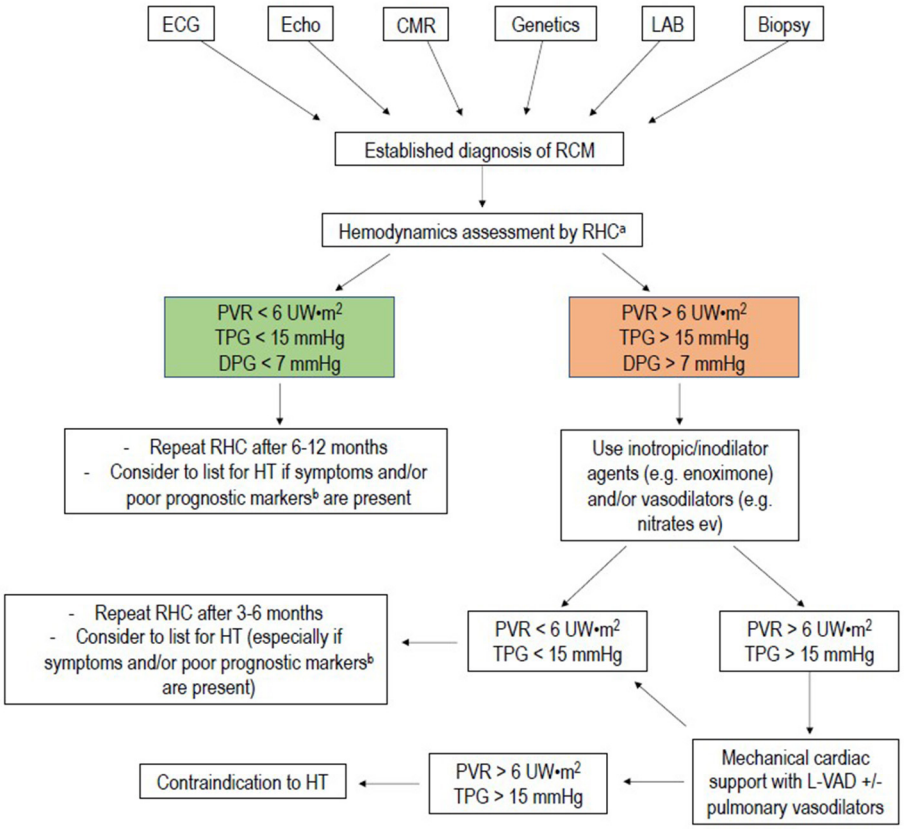

FIGURE 6 | Flow-chart of management of pediatric restrictive cardiomyopathy. RHC, right heart catheterization; PVR, pulmonary vascular resistance; mPAP, mean pulmonary artery pressure; TPG, transpulmonary gradient; DPG, diastolic pulmonary gradient; HT, heart transplantation. [a]RHC is usually indicated for all patients referred for heart transplantation. RHC in addition, may help to stratify prognosis in patients in NYHA class I to II: severe pulmonary hypertension and low cardiac output can develop even in absence of symptoms, leading to late referral for heart transplantation. When at least one of the prognostic indicators for RCM is present, it may be advisable to refer the patient to a tertiary center for HT evaluation. [b]Poor prognostic markers are: pulmonary congestion, myocardial ischemia, severe left atrial dilation, male gender, reduction of left ventricular fractional shortening, increased left ventricular walls thickness.

Whole-body scintigraphy or SPECT with bone-seeking tracers [(99mTc)-labeled bisphosphonate compounds: pyrophosphate (PYP); 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD), and hydroxydiphosphonate (HDP)] can reveal amyloid deposits (especially in ATTR subtype) in the heart, as well as PET with [^{18}F] FDG can detect inflammatory cells in some pathological processes such as cardiac sarcoidosis. However, due to the rarity of the aforementioned diseases and due to the concern for radiation exposure in childhood, nuclear imaging has very limited applications in the diagnostic work-up of pediatric RCMs.

Cardiac catheterization is usually not necessary for RCM diagnosis, but it can be useful to distinguish between restrictive and constrictive physiology and to determine the severity of the diastolic dysfunction by directly measuring the filling pressures of both ventricles (**Figure 6**). In RCM left ventricular end-diastolic pressures are usually higher than right end-diastolic pressure, whereas are equal or very nearly equal in CP. Furthermore, specular discordance between RV and LV peak systolic pressures during inspiration are typical of CP, with an increase in RV pressure occurring during peak inspiration, when LV pressure is lowest. Cardiac catheterization can also reveal the presence of PH, detect the presence of elevated pulmonary vascular resistance, evaluate the cardiac index and test for pulmonary vasculature reactivity.

Plasma levels of natriuretic peptides can be helpful in the diagnostic pathway of RCM, especially in the differential diagnosis with CP. A study of 49 adults (20 with RCM and 29 with CP) showed that median plasma NT-proBNP was 1,775 (208–7,500) pg/ml in those with RCM vs. 124 (68–718) pg/ml in those with CP ($P = 0.001$) (66). Specific etiologies may require additional laboratory exams such as angiotensin converting enzyme dosage in sarcoidosis, complete blood count to establish eosinophilia in HESs, serum iron concentrations, total iron-binding capacity, and ferritin levels in hemochromatosis, alpha-galactosidase activity, and lyso-Gb3 levels in AFD, immunoglobulin free light chain testing, and serum and urine immunofixation in AL amyloidosis.

The endomyocardial biopsy can be valuable in doubtful cases, when non-invasive tests are inconclusive. Unfortunately, in idiopathic RCM it often demonstrates non-specific findings such

TABLE 1 | Differential diagnosis of constrictive pericarditis and restrictive cardiomyopathy.

	Constrictive pericarditis	Restrictive cardiomyopathy
Clinical examination	Kussmaul's sign, usually present	Kussmaul's sign, may be present
	Pulsus paradoxus (may be present)	Pulsus paradoxus (infrequent)
	Pericardial knock	S3; Systolic murmur due to mitral and tricuspidal regurgitaion
Echocardiogram		
Pericardial appearance	Thickened/bright	Normal
Atrial size	Minor enlargement	Major enlargement
Septal motion	Respiratory shift	Normal
Mitral inflow respiratory variation	Usually present (>25%)	Absent
TDI septal S' wave, cm/s	>5	<5
Speckle tracking	↓ Circumferential strain	↓ Radial and longitudinal strain
Biomarkers		
NT-proBNP	Normal or slightly abnormal	Abnormal
Cardiac catheterization		
Right and left ventricular end-diastolic pressures comparison	Equal or ≤5 mm Hg	Usually left > right
Dip-plateau waveform	Typically present	Can be present
CT scan/MRI		
Pericardial thickening	Present	Absent

as myocyte hypertrophy, interstitial and/or endocardial fibrosis. Furthermore, periprocedural risks in fragile affected children should be considered (50).

Restrictive cardiomyopathy should be distinguished from CP, since the two diseases have different treatments and outcomes. In some cases—namely, radiation induced cardiac disease—restriction and constriction may coexist in the same patient, making final diagnosis even more challenging. In **Table 1** the main instrumental features of each condition are summarized.

MANAGEMENT

Current medical therapy for RCM is primarily supportive and is in large part limited to diuretics in patients with signs and symptoms of systemic or pulmonary venous congestion. The International Society for Heart and Lung Transplantation (ISHLT) guidelines for the management of pediatric heart failure published in 2014 recommend in class I the diuretic therapy to establish a clinically euvolemic state, with a close monitoring of renal function and blood pressure during initiation and up-titration (67). Diuretic therapy reduces signs of systemic congestion, with beneficial effect on symptoms as dyspnea, fatigue, peripheral edema, and cough. However, excessive diuresis should be avoided because these patients are sensitive to alterations in preload. Furthermore, diuretics can mask an underlying PH or increased biventricular filling pressures, therefore this aspect must be considered before performing a hemodynamics invasive assessment (particularly prior to candidacy to heart transplantation). Restrictive cardiomyopathy has a unique pathophysiology: small ventricular cavity dimensions and rapid increase of filling pressures significantly compromise stroke volume (according to pressure-volume loop). Therefore, cardiac output is strongly influenced by heart rate, which must be kept at relatively high values to guarantee adequate systemic perfusion. For these reasons β-blockers and calcium channel blockers are not currently recommended in pediatric RCM, unless for a different indication (67). Angiotensin converting enzyme inhibitors and angiotensin receptor blockers may be considered if coexisting systemic arterial hypertension is present (class IIb recommendation). Similarly, digoxin (unless for rate control of atrial arrhythmias), intravenous inotropes (such as dopamine, dobutamine, and epinephrine), and pulmonary vasodilators (prostaglandins and endothelin receptor antagonists to treat secondary PH) are generally not recommended (Class III).

Although atrial thrombosis has been linked to atrial fibrillation, abnormal hemodynamics, and to a possible hypercoagulable state in adults with RCM, dedicated studies in pediatric populations are lacking (68). The incidence of intracardiac thrombus in the reviewed literature ranges from 0 to 42%, with rates of embolism between 12 and 33%. The risk of embolism appears to be much greater in children with RCM than to DCM; therefore, antithrombotic or anticoagulation therapy should be considered at the time of diagnosis (47, 69). However, there are no studies comparing the effectiveness of antiplatelet agents, vitamin K antagonists or enoxaparin in preventing embolism in children with RCM (70).

Conduction system disease recognize different etiologies, such as ischemic injury of the atrio-ventricular node and His-Purkinje system, mechanical stretching due to atrial and ventricular dilation or genetically determined mechanisms (71–73). In addition, prolonged PR interval and wide QRS complex at ECG were associated with acute cardiac event (49). The 2014 ISHLT guidelines recommend in Class I permanent PM for advanced second- or third-degree atrioventricular block associated with ventricular dysfunction. On the basis of available data, the presence of conduction system disease should trigger increased surveillance through baseline ECG and ECG Holter monitoring with ST-segment analysis together with a routine screening for research of clinical (chest pain or syncope) and instrumental ischemia (ST-segment variations). Some Authors suggest in the presence of PR prolongation, QRS widening, and left bundle-branch block particular attention and to consider prophylactic pacing, eventually as part of an implantable-cardioverter defibrillators (ICD) system (48). However, it should be noticed that there are no studies documenting the efficacy of defibrillator systems in large pediatric cohorts, and case to case evaluation, assessing the individual risk factors (including the specific etiology) should be carried out, in order to avoid inappropriate and detrimental therapies (49). At moment, without unanimous criteria, ICD should be considered in the subset of pediatric RCM patients with evidence of ischemia and

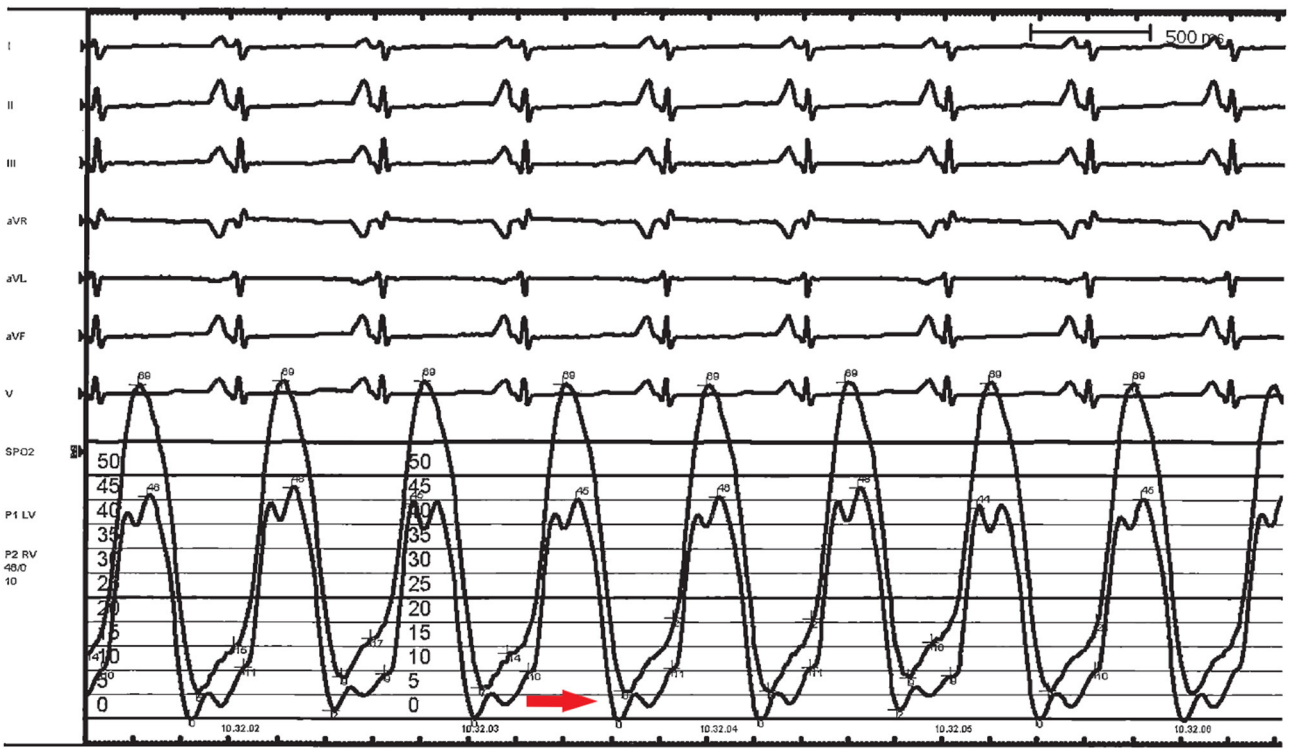

FIGURE 7 | Left ventricular (LV) and right ventricular (RV) hemodynamic pressure tracings in a 3-year-old girl affected by restrictive cardiomyopathy. End-diastolic filling pressures are elevated, with LV values higher than those in RV, and a "square root" sign is present on both tracings (red arrow).

ventricular arrhythmia, where also β-blockers may be beneficial (48). In a single center experience of pediatric patients with RCM, 40% of them with an ICD or PM, device therapies were relatively rare and inappropriate therapies were exceedingly rare (6).

These gaps in evidence are partly to ascribe to the low prevalence of RCM and its poor prognosis, with half patients dying or being referred to cardiac transplantation within 3 years from the diagnosis (74). Medical treatment has not shown any significant long term benefit and cardiac transplantation is the only effective therapy with survival rates between 70 and 60% at 5 and 10 year, respectively, in children listed for heart transplant (75). Orthotopic heart transplantation is preferred to heart-lung or heterotopic heart transplant and has a better survival rate than the other two options (76). In 2012 Singh et al. analyzed 1,436 children <18 years of age with a diagnosis of cardiomyopathy listed for heart transplant in the United States between 2004 and 2010, of which 167 with RCM. In adjusted analysis, children with non-DCM (83%) had a higher risk of wait-list mortality only if supported by a ventilator at listing. Post-transplant 30-days and at 1-year survival were similar between children with dilated and non-DCM ($p = 0.17$) (77). In another cohort of children with RCM from the American Pediatric Cardiomyopathy Registry database, about two thirds of children had a pure RCM phenotype, and the rest had a mixed RCM/HCM phenotype. Rate of survival at 5 year was 20 and 28%, respectively, but patients with pure RMC phenotype underwent heart transplantation more frequently (58 vs. 30%) (5).

The majority of deaths in children awaiting heart transplantation is due to progressive heart and multi-organ failure. Hence, in this context, mechanical circulatory support (MCS), as bridge to transplantation or candidacy, may be a precious weapon. At the moment the experience with MCS in children is quite limited, and this is particularly true for RCM. The most used pediatric long-term support device, is the pneumatically driven, pulsatile EXCOR® Berlin Heart, with a variety of pump sizes, covering almost all pediatric patients, and the only long-term device for neonates and infants approved in Europe and USA. Few case series of successful bridging to cardiac transplantation with the Berlin EXCOR® left ventricular assist device (LVAD) have been described (78, 79). Conventional LV apical cannulation of a non-compliant left ventricle often results in insufficient drainage and poor pump performance, with residual high left atrial pressures and consequent pulmonary congestion. Left atrial cannulation (such as in EXCOR® Berlin Heart) is therefore an interesting option in patient with small ventricular cavity, preserved systolic function, and enlarged atria (80). A recent review of the American registry of EXCOR® Berlin Heart implantation in pediatric patients affected by RCM showed a survival rate of 50%, which is significantly less than that of the overall EXCOR ® pediatric population (75%) (81). Primary

causes of death included stroke, infection, acidosis, multisystem organ failure, and bleeding. It is of note that these patients tended to be sicker than the whole population (INTERMACS class 1 and with ECMO support) and this can explain at least partly, the worst outcome. In summary, long-term MCS implantation is a high-risk procedure that can be considered in advanced stages of the disease as bridge to transplantation or to candidacy by improving hemodynamics, with the reduction of post-capillary PH. Further studies are needed to determine the best timing for the procedure and the best anticoagulant strategy to reduce the risk of thromboembolic events that are the main cause of adverse outcome in these patients.

Considering all these major concerns about the poor efficacy of medical treatment, the identification of the right time to list for heart transplantation a patient affected by RCM during the clinical follow-up becomes both challenging and crucial. Although some clinical and instrumental factors such as pulmonary congestion at diagnosis, severe left atrial dilation, or increased ventricular wall thickness have been identified as potential predictors of poor prognosis, there are no established criteria for listing and the decision is often dependent on individual experienced centers (82). A multi-modal instrumental approach is essential, particularly based on a regular assessment of right heart hemodynamics with or without use of inotropic agents and vasodilators, to detect at the proper time the development of irreversible PH. Some pediatric institutions consider the development of PH an indication for listing, regardless of heart failure symptoms. Accordingly, right heart catheterization is mandatory at first evaluation, since up to 50% of patients have PH at diagnosis (47, 83). The flow-chart illustrated in **Figure 7** summarizes the experienced approach for pediatric RCM, developed in our tertiary center, with a dedicated program for pediatric cardiomyopathies and pediatric heart transplantation.

CONCLUSION

Pediatric RCM is a rare disorder, due to a large heterogeneous group of causes. As a result of its poor prognosis, RCM contributes disproportionately to mortality in children with cardiomyopathy, being heart transplantation the only effective treatment. Therefore, early referral to a third-level cardiomyopathy center is warranted for careful observation, to avoid the development of irreversible PH and to avoid a delay in listing for heart transplantation when indicated.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Different Pattern of Cardiovascular Impairment in Methylmalonic Acidaemia Subtypes

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Methylmalonic acidaemia (MMA) has been reported to be associated with cardiovascular involvement, especially for the combined type with homocystinuria. We have screened 80 control subjects and 99 MMA patients (23 isolated type and 76 combined type) using electrocardiograph and echocardiography. 32 cases (34%) of ECG changes were found including sinus tachycardia ($n = 11$), prolonged QTc interval ($n = 1$), I-degree atrioventricular block ($n = 1$), left axis deviation ($n = 5$) and T wave change ($n = 14$). By echocardiography, 8 cases of congenital heart disease were found in 4 combined MMA patients (5.3%) including ventricular septal defect ($n = 2$), atrial septal defect ($n = 3$), patent ductus arteriosus ($n = 1$) and coronary artery-pulmonary artery fistula (n =2). Pulmonary hypertension ($n = 2$) and hypertrophic cardiomyopathy ($n = 1$) in combined subtype were also noted. Moreover, echocardiographic parameters were analyzed by multiple regression to clarify the influence of different subtypes on cardiac function. It was found that the left ventricular mass index (LVMI) was significantly reduced only in combined subtype [$R = -3.0$, 95%CI (-5.4, -0.5), $P = 0.017$]. For left ventricle, the mitral E' velocity was significantly reduced [isolated type: $R = -1.8$, 95%CI (-3.3, -0.4), $P = 0.016$; combined type: $R = -2.5$, 95%CI (-3.5, -1.5), $P < 0.001$], the global longitudinal strain (GLS) was the same [isolated type: $R = -1.4$, 95%CI (-2.3, -0.4), $P = 0.007$; Combined type: $R = -1.1$, 95%CI (-1.8, -0.4), $P = 0.001$], suggesting weakened left ventricular diastolic and systolic functions in both subtypes. For right ventricle, only in combined subtype, the tricuspid E' velocity was significantly reduced [$R = -1.4$, 95%CI (-2.6, -0.2), $P = 0.021$], and the tricuspid annular plane systolic excursion (TAPSE) was the same [$R = -1.3$, 95%CI (-2.3, -0.3), P=0.013], suggesting impaired right ventricular systolic and diastolic function. In conclusion, isolated and combined types showed different pattern of cardiac dysfunction, specifically the former only affected the left ventricle while the latter affected both ventricles. And it is necessary to perform echocardiographic screening and follow up in both MMA subtypes.

Keywords: methylmalonic acidaemia, homocystinuria, cobalamin C type, cardiac dysfunction, congenital heart disease, pulmonary arterial hypertension

INTRODUCTION

Methylmalonic acidaemia (MMA) is a class of diseases due to various inherited autosomal recessive gene defects, which result in impaired function of methylmalonyl-CoA mutase (MCM) or impaired intracellular cobalamin (Cbl) transport and processing (1, 2). Clinically, MMA can be divided into two types, isolated MMA and combined MMA which is also called MMA with homo-cystinuria (MMA/HCY). The former is due to MCM defects or Cbl A/B defects which only cause deficient adenosylcobalamin (AdoCbl) within mitochondrion, while other Cbl defects can also affect methylcobalamin (MeCbl) synthesis and results in the latter (**Figure 1**). In China, combined type is the most common type, and CblC defect accounts for most cases with related gene identified as *MMACHC* (3), which is located on chromosome 1p and responsible for CNCbl decyanase.

This disease has a broad spectrum of clinical manifestations. Most patients suffer from nervous system impairment and extranervous system involvement. Currently, cardiovascular involvement has begun to draw attention as an increasing number of cases have been reported in combined type, especially CblC patients. The first CblC patient with cor pulmonale as a complication was reported by Brandstetter et al. in 1990 (4). Profitlich et al. (5) conducted a retrospective study to analyse echocardiographic data in ten CblC patients and found that half of them had structural heart defects. In some patients, the sudden onset of pulmonary arterial hypertension (PAH) or renal hypertension can be a trigger of heart failure and progress into a life-threatening event (6–10).

Cardiomyopathy has been observed in many inherited metabolic diseases, such as Anderson-Fabry disease (11), as cardiac energetic impairment can play a causal role in cardiac dysfunction and vulnerability (12, 13). Both types of MMA patients have also shown manifestations such as dilated or hypertrophic cardiomyopathy.

Currently, no studies have investigated the cardiac function of MMA patients in the stable stage. Echocardiography has been a useful and non-invasive tool to measure cardiac structure, function and haemodynamic. Tissue Doppler imaging can provide an assessment of myocardial systolic and diastolic function in both ventricles (14), and a novel technique, speckle-tracking echocardiography (STE), can provide an accurate assessment of myocardial deformation and detect preclinical myocardial dysfunction when EF is normal (15–18).

Hence, we organized a screening of the cardiovascular system to assess cardiac function and clarify the incidence of congenital heart disease (CHD), cardiomyopathy and PAH in MMA patients and explore the difference between the subtypes.

METHODS AND MATERIALS

Population

From April 2019 to August 2019, we organized cardiovascular system screenings in three areas of China (the provinces of Liaoning, Shandong and Anhui) for MMA patients who were recruited at local tertiary hospitals (Shengjing Hospital of China Medical University, Jinan Maternity and Child Care Hospital, and Anhui Women and Child Health Care Hospital). During consultation, clinical information was collected, such as the diagnosis (isolated or combined type) according to blood tandem mass spectrometry and urinary gas chromatography, genetic diagnosis, and others.

The control group consisted of age- and gender-matched children who were referred for a pediatric cardiology

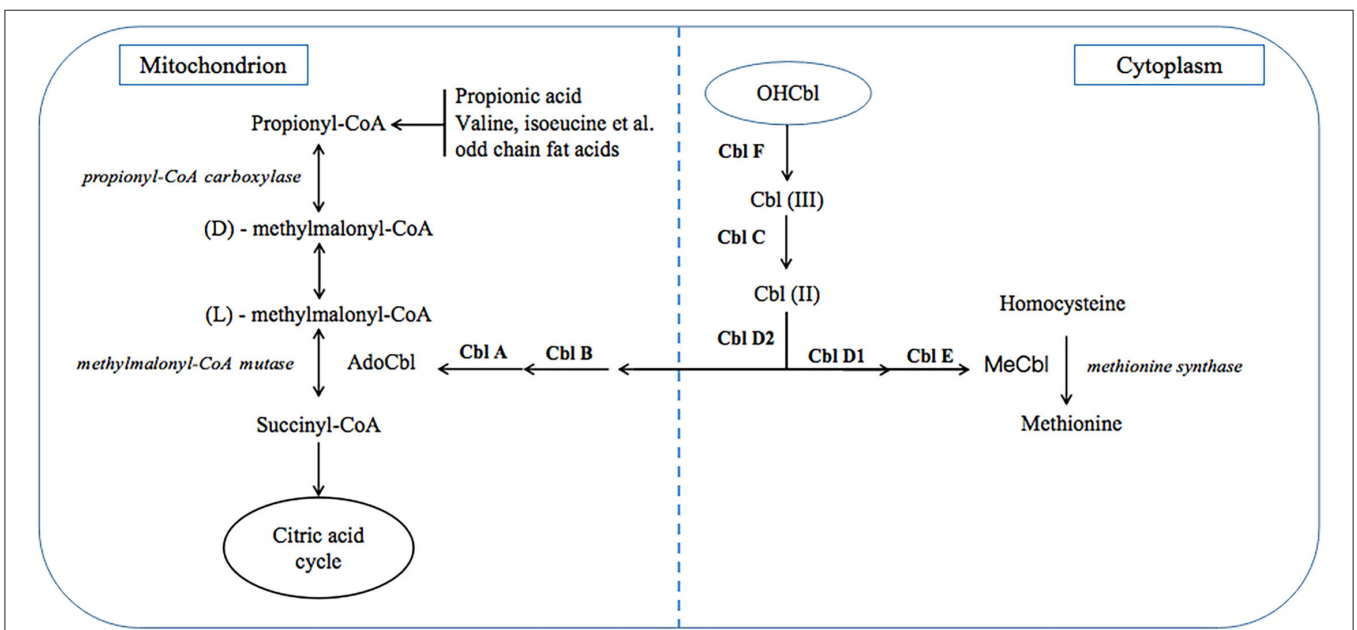

FIGURE 1 | Metabolic pathway of cobalamin and MMA.

consultation at XinHua Hospital. The inclusion criteria included minor clinical symptoms (innocent murmur) and normal results on electrocardiography (ECG) and conventional echocardiography. The exclusion criteria included children with a disease or undergoing a treatment that may affect heart function.

Clinical Assessments and ECG

Height and weight were measured in all subjects. For subjects aged over 3 years old, blood pressure (BP) and heart rate (HR) was measured during rest using an Omron HBP-1,300 professional blood pressure monitor (Omron Healthcare, Guangzhou, CHINA) (19) in the supine position.

Standard 12-lead ECG was performed with the subject in the supine position, and the results were analyzed by a professional medical officer.

Conventional Echocardiography and STE

Transthoracic echocardiography examinations were performed by a single senior echocardiologist using a CX 50 ultrasound system (Philips Healthcare, Andover, USA). Image acquisition was conducted including M-mode, spectral Doppler flow, tissue Doppler imaging (TDI) (20, 21), and three to five cardiac-cycle loops in apical four-, three-, and two-chamber views for STE analysis (16). S 8-3 and S 5-1 probes (Philips Healthcare, Andover, USA) were used according to the age and weight of the subject.

For each subject, the velocity of tricuspid regurgitation (V_{TR}) was quantified if present. The pulmonary arterial systolic pressure (PASP) was calculated by the modified Bernoulli equation, and PAH was diagnosed if PASP >40 mmHg (22).

The left ventricular internal diastolic diameter (LVIDD) was measured using M-mode in the parasternal short-axis view. The LV mass (LVM) was calculated by Devereux's formula (23), and the LVM index (LVMI) (24) was calculated by dividing the LVM by height∧2.7. LV fractional shortening (FS) was calculated as (LVIDD-LVIDS)/LVIDD. The LV ejection fraction (LVEF) was calculated by the cubed method.

All of the following parameters were measured three times in independent cardiac cycles and averaged: the LV early and late diastolic mitral inflow velocity (LV E/ LV A), and peak early diastolic velocity (LV E') at the lateral segment of the mitral annulus; the mean velocity of circumferential fiber shortening corrected for cardiac frequency (mVCFc) and myocardial performance index (MPI); the right ventricular (RV) peak early diastolic velocity (RV E') and early systolic velocity (RV S') measured at the lateral segment of the tricuspid annulus; and the tricuspid annular plane systolic excursion (TAPSE) measured along its longitudinal plane from end-diastole to end-systole.

STE analysis was performed using commercial QLAB version 10.5 software (Philips Healthcare, Andover, USA). The region of interest was anchored as the endocardium in end-diastole, and their longitudinal strain during a heartbeat was detected by the software. The global longitudinal strain (GLS) of the left ventricle was then calculated from the 17-segment model.

TABLE 1 | Baseline characteristics of control, isolated MMA, and MMA/HCY groups.

Characteristics	Control subjects (*N* = 80)	Isolated MMA (*N* = 23)	MMA/HCY (*N* = 76)	*P**
Age (year)	4.1 ± 2.7	6.0 ± 6.6	3.5 ± 2.9	0.226
Male	46	15	38	0.265
Weigh Z score	0.4 ± 1.2	−0.3 ± 1.5	−0.3 ± 1.2	0.001
Height Z score	0.5 ± 1.4	−0.2 ± 1.4	0.2 ± 1.3	0.126
BMI Z score	0.1 ± 1.6	−0.2 ± 1.7	−0.6 ± 1.5	0.003
SBP (mmHg)	97.7 ± 11.2	102.2 ± 18.3	97.5 ± 10.3	0.733
DBP (mmHg)	55.6 ± 10.4	61.6 ± 11.8	58.2 ± 9.8	0.079
HR (bpm)	94.4 ± 13.7	107.6 ± 22.4	111.7 ± 19.5	<0.001

Data are expressed as the mean ± SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

**According to t-test or Kruskal-Wallis test for continuous data and chi-squared test for categorical data, P <0.05 is considered significantly different between groups.*

Statistical Analysis

The analysis was performed with EmpowerStats software (version 3.0) and Graphpad Prism (12.0). Continuous variables are presented as the mean ± SD, and categorical variables are presented as frequencies or proportions. To minimize the influence of age, height, weight and body mass index (BMI) were converted to a Z score using the Growth Charts^{UK-WHO} application (version 2.0.1) with references, including the Neonatal and Infant Close Monitoring Growth Chart, the UK WHO 0–4 years' growth chart, and the UK Growth chart (2–18). Comparisons between the two groups were performed using the *t*-test or Kruskal-Wallis rank-sum test for continuous data and chi-squared test for categorical data. Multiple regression analysis was used to identify echocardiography variables affected by the exposure of different types; model I was adjusted for gender, age, and BMI Z score, and model II was adjusted for gender, age, BMI Z score, BP, and HR. A p value < 0.05 was considered statistically significant.

RESULTS

Population and Baseline Characteristics

A total of 99 patients and 80 control subjects were recruited in this study, with 23 isolated MMA and 76 combined MMA; of them, 84 patients underwent genetic testing and variant identification, and all identified combined MMA patients were the CblC type (**Supplementary Figure 1** and **Table 1**). The basic characteristics were summarized in **Table 1**. No difference was observed in age, gender, height Z score or BP between three groups. Compared to the controls, the weight Z score (0.4 ± 1.2 vs. −0.3 ± 1.5 vs. −0.3 ± 1.2, $P = 0.001$) and BMI Z score (0.1 ± 1.6 vs. −0.2 ± 1.7 vs. −0.6 ± 1.5, $P = 0.003$) were decreased in both types of MMA patients, which was consistent with their limited developmental state. The heart rate of patients was elevated compared with that of controls (94.4 ± 13.7 vs. 107.6 ± 22.4 vs. 111.7 ± 19.5, $P < 0.001$).

TABLE 2 | Echocardiographic parameters of isolated MMA and MMA/HCY groups.

Parameters	Isolated MMA ($N = 21$)	MMA/HCY ($N = 73$)
P-R interval (ms)	124.4 ± 20.1	110.6 ± 18.1
QRS (ms)	77.9 ± 9.5	73.1 ± 8.5
QTc interval (ms)	312.0 ± 37.6	298.6 ± 31.9
QRS axis (degree)	63.7 ± 24.1	58.8 ± 39.7

Data are expressed as the mean ± SD.

Electrocardiography

On ECG, 94 patients have been examined and detailed parameters were listed in **Table 2**, which were within normal range in both groups. Although no clinically significant changes were found such as premature ventricular contraction, we have noted 11 cases of sinus tachycardia, 1 prolonged QTc interval, 1 I-degree atrioventricular block, 5 cases of left axis deviation, and 14 cases of T wave change such as higher T waves or flat T wave tops with notch.

Echocardiographic Variables

Table 3 demonstrated the echocardiographic variables in three groups. Among indexes reflecting left ventricular size, the LVIDD and LVIDS were much smaller in combined type compared to control group and isolated type (LVIDD: 34.7 ± 4.8 vs. 35.1 ± 8.4 vs. 30.9 ± 6.0, $P < 0.001$; LVIDS: 22.2 ± 3.2 vs. 22.2 ± 6.0 vs. 19.8 ± 4.1, $P < 0.001$), indicating a hypogenetic heart in this group, while the LVMI showed no difference between them. The LVEF was within normal range in three groups, although MMA patients had higher LVEF than controls (69.0 ± 9.2 vs. 74.9 ± 4.4 vs. 73.6 ± 4.2, $P < 0.001$). The other two traditional systolic function indexes, LVFS and mVCFc, were not significantly different among three groups, while GLS, a new sensitive index, was reduced in MMA patients (22.7 ± 1.8 vs. 20.3 ± 2.6 vs. 21.3 ± 1.9, $P < 0.001$). This indicator suggested impaired myocardial systolic function. The ratio of LV E/A, an index reflecting LV diastolic function, showed significant reduction in patients compared with control subjects (1.6 ± 0.3 vs. 1.5 ± 0.3 vs. 1.4 ± 0.2, $P = 0.002$), which was consistent with the LV E' velocity (16.0 ± 3.1 vs. 14.5 ± 2.5 vs. 13.1 ± 3.3, $P < 0.001$).

For the right ventricle, the TAPSE, an index of RV systolic function, showed a significant reduction in combined type compared to the others (19.0 ± 3.2 vs. 19.6 ± 4.0 vs 17.2 ± 3.2, $P < 0.001$), while another systolic index RV S' showed a different tendency (12.7 ± 2.2 vs. 14.0 ± 2.5 vs. 12.9 ± 1.8, $P = 0.033$). As for diastolic function, there was no significant difference in RV E' among three groups.

Multiple Regression Analysis

As results showed above, the changing tendency of cardiac function among three groups can be quite conflicting according to different echocardiographic variables. Thus, we have done multiple regression analysis to rule out other factors such as age and gender to clarify the influence of exposure of MMA subtypes on cardiac function.

TABLE 3 | Echocardiographic variables of control, isolated MMA, and MMA/HCY groups.

	Echocardiographic variables	Control subjects ($N = 80$)	Isolated MMA ($N = 23$)	MMA/HCY ($N = 76$)	P^*
LV	LVIDD (mm)	34.7 ± 4.8	35.1 ± 8.4	30.9 ± 6.0	<0.001
	LVIDS (mm)	22.2 ± 3.2	22.2 ± 6.0	19.8 ± 4.1	<0.001
	IVS (mm)	6.4 ± 1.1	6.7 ± 1.8	6.2 ± 1.3	0.269
	IVS/D	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.3	0.369
	EF (%)	69.0 ± 9.2	74.9 ± 4.4	73.6 ± 4.2	<0.001
	FS (%)	35.9 ± 4.6	37.1 ± 3.9	35.9 ± 3.6	0.733
	LVM (g)	34.3 ± 13.5	41.7 ± 36.0	27.2 ± 17.9	<0.001
	LVMI ($g/m^{2.7}$)	31.7 ± 8.9	31.9 ± 10.4	29.5 ± 9.6	0.100
	E/A ratio	1.6 ± 0.3	1.5 ± 0.3	1.4 ± 0.2	0.002
	E' (cm/s)	16.0 ± 3.1	14.5 ± 2.5	13.1 ± 3.3	<0.001
	mVCFc (sec^{-1})	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	0.841
	MPI	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.360
	GLS (%)	22.7 ± 1.8	20.3 ± 2.6	21.3 ± 1.9	<0.001
RV	E'	14.8 ± 3.0	15.9 ± 4.4	14.5 ± 3.5	0.509
	S'	12.7 ± 2.2	14.0 ± 2.5	12.9 ± 1.8	0.033
	TAPSE	19.0 ± 3.2	19.6 ± 4.0	17.2 ± 3.2	<0.001

Data are expressed as the mean ± SD. LV, left ventricle; RV, right ventricle; LVIDD, left ventricular internal diastolic diameter; LVIDS, left ventricular internal systolic diameter; IVS, interventricular systolic septum; IVS/D, ratio of interventricular systolic and diastolic septum; EF, ejection fractio; FS, fractional shortening; LVM, left ventricular mass; LVMI, left ventricular mass index; E/A, ratio of early and late diastolic mitral inflow velocity; E', peak early diastolic velocity; S', peak early systolic velocity; mVCFc, mean velocity of circumferential fiber shortening; MPI, myocardial performance index; GLS, global longitudinal strain; TAPSE, tricuspid annular plane systolic excursion.

**According to t-test or Kruskal-Wallis test for continuous data, $P < 0.05$ is considered significantly different between groups.*

Figures 2, **3** showed the regression coefficients and 95% confidence intervals, detailed values were in **Supplementary Table 2**. According to model III, LVIDD, and LVIDS both diminished due to exposure of isolated or combined MMA (LVIDD: isolated [$R = -1.5$, 95%CI (−2.9, −0.1), $P = 0.036$] combined [$R = -2.0$, 95%CI (−3.0, −1.1), $P < 0.001$]; LVIDS: isolated [$R = -1.7$, 95%CI (−2.4, −1.0), $P < 0.001$] combined [$R = -1.2$, 95%CI (−1.9, −0.4), $P = 0.002$)], while LVMI showed significant reduction only in combined type [$R = -3.0$, 95%CI (−5.4, −0.5), $P = 0.017$]. LVEF still remained elevated [isolated: $R = 5.6$, 95%CI (2.0, 9.1), $P = 0.002$; combined: $R = 4.2$, 95%CI (1.7, 6.6), $P = 0.001$], and GLS reduced [isolated: $R = -1.4$, 95%CI (−2.3, −0.4), $P = 0.007$; combined: $R = -1.1$, 95%CI (-1.8, −0.4), $P = 0.001$] in both MMA groups. Although the ratio E/A showed no more difference, the LV E' velocity still showed significant reduction in both MMA groups [isolated: $R = -1.8$, 95%CI (−3.3, −0.4), $P = 0.016$; combined: $R = -2.5$, 95%CI (−3.5, −1.5), $P < 0.001$]. As for right ventricle, only combined type had significant reduction in TAPSE [$R = -1.3$, 95%CI (−2.3, −0.3); $P = 0.013$], and RV E' [$R = -1.4$, 95%CI (−2.6, −0.2); $P = 0.021$].

Clinical Cardiovascular Involvement

During our echocardiography examination, 8 combined MMA patients with cardiovascular involvement were

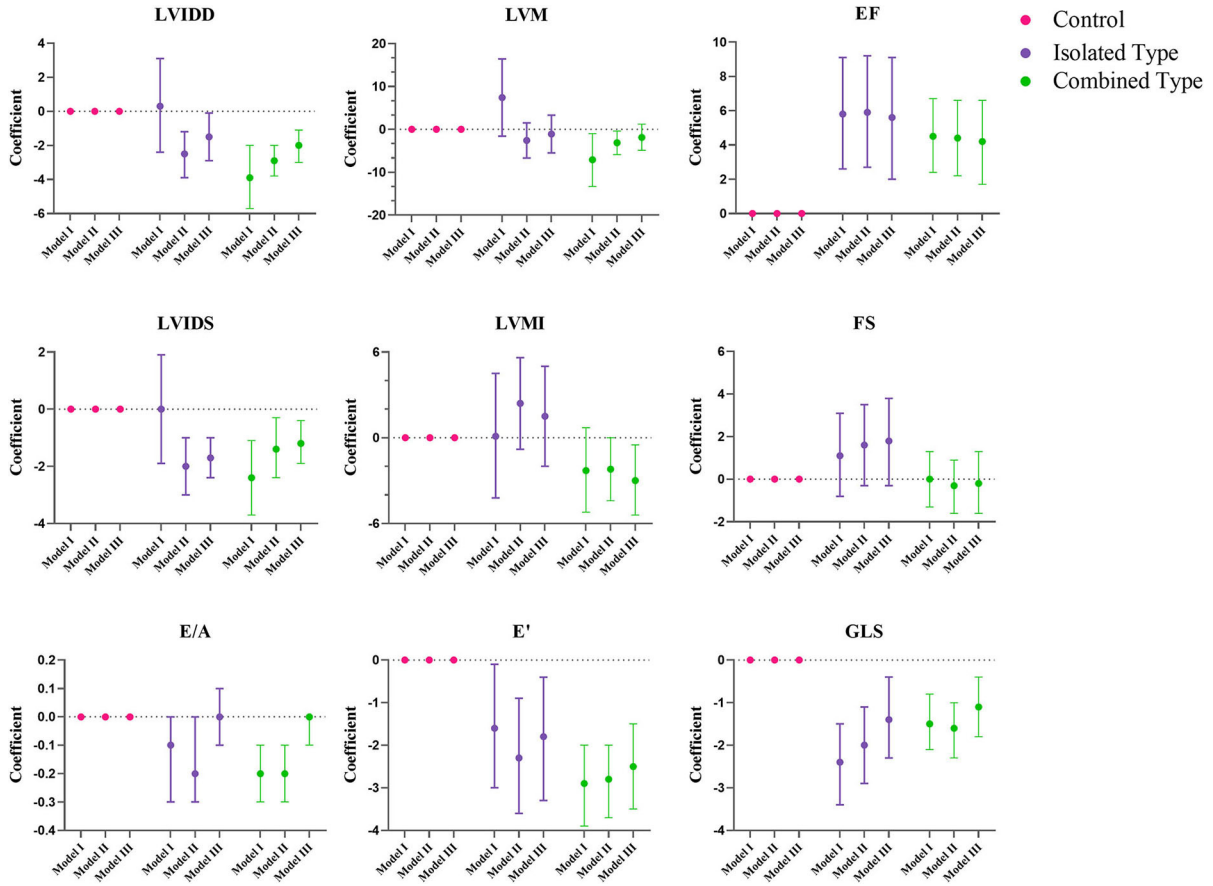

FIGURE 2 | Multiple regression analysis of left ventricular echocardiographic variables between control, isolated MMA and combined MMA patients. Multiple regression analysis was used with control group as reference. Model I was not adjusted, model II was adjusted for age, gender and BMI Z score, and model III was adjusted for age, gender, BMI Z score, blood pressure and heart rate. LVIDD, left ventricular internal diastolic diameter; LVIDS, left ventricular internal systolic diameter; EF, ejection fraction; FS, fractional shortening; LVM, left ventricular mass; LVMI, left ventricular mass index; E/A, ratio of early and late diastolic mitral inflow velocity; E', peak early diastolic velocity; GLS, global longitudinal strain.

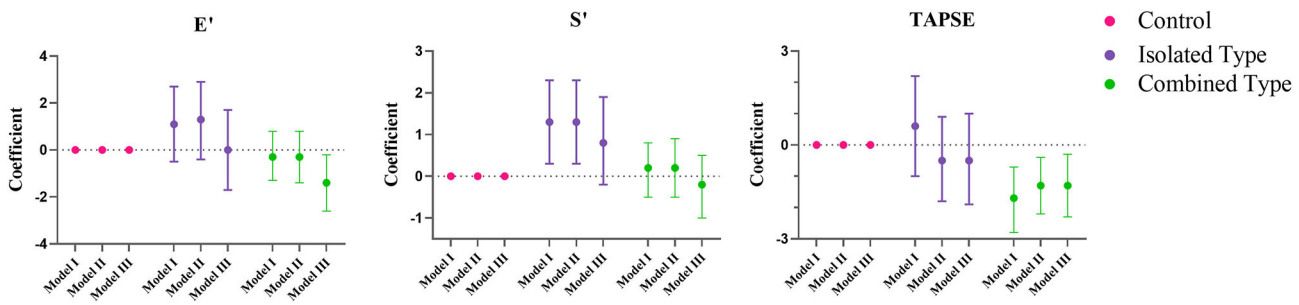

FIGURE 3 | Multiple regression analysis of right ventricular echocardiographic variables between control, isolated MMA and combined MMA patients. Multiple regression analysis was used with control group as reference. Model I was not adjusted, model II was adjusted for age, gender and BMI Z score, and model III was adjusted for age, gender, BMI Z score, blood pressure and heart rate. E', peak early diastolic velocity; S', peak early systolic velocity; TAPSE, tricuspid annular plane systolic excursion.

noted, accounting for 10.5% of this population, and their detailed information was listed in **Table 4**. There were eight cases of CHD [including two ventricular septal defects (VSD), three atrial septal defects (ASD), one patent ductus arteriosus (PDA), and two coronary artery-pulmonary artery fistulas (CA-PAF)], one case of pulmonary hypertension, and one case of hypertrophic cardiomyopathy.

Patient 1 had a muscular VSD with a diameter of 1.8 mm and a CA-PAF. Patient 2 had a ASD and a PDA. Patient 3 had

TABLE 4 | Clinical information of combined MMA patients with cardiovascular involvement.

Patient	1	2	3	4	5	6	7	8
Gender	Female	Female	Male	Male	Female	Male	Male	Male
Age at onset	1 month	1 month	NA	NA	Birth	3 months	6 years	4 years
Age at diagnosis	2 months	4 months	15 days	20 days	1 month	2 years and 3 months	6 years	4 years
MMACHC variant	c.567dupT/ c.567dupT	c.609G> A/c.658_660delAAG	NA	c.609G>A/c.609G>A	c.609G>A/c.616C>T	c.609G>A/c.80A>G	c.80A>G/ c.217C>T	c.609G>A/c.80A>G
Cardiovascular involvement	Muscular VSD/CA-PAF	ASD/PDA	ASD/VSD/LV enlargement	CA-PAF	LV hypertrophy	PAH/heart failure	Systemic hypertension/heart failure	PAH
Other complications	/	/	/	/	/	CKD I/anemia	Haematuria/ proteinuria/ anemia	AHS/hypothyroidism

ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; CA-PAF, coronary artery-pulmonary artery fistula; AHS, alveolar haemorrhagic syndrome; CKD, chronic kidney disease; PAH, pulmonary arterial hypertension; NA, not available.

two ASDs (2.5 mm and 3.5 mm, respectively) and a VSD, which were responsible for the enlargement of the LV and required an interventional operation for defect closure. Patient 4 had a CA-PAF, and his left coronary artery was slightly enlarged, with a diameter of 3.13 mm. Patient 5 had a relatively thick interventricular septum for her age as the IVS during systolic phase was 7.16 mm and the Z score was +1.52.

Patient 6 presented at 3 months of age with jaundice and failure to thrive and had shown progressive cyanosis since 1.5 years of age. He was admitted at 2 years and 3 months of age due to oedema and worsening cyanosis. The CblC type was diagnosed genetically with variants (c.609G>A/c.80A>G). Echocardiography revealed PAH (WHO FC IV) with a PASP of 96 mmHg and a significantly dilated right ventricle. With treatment with PAH-targeted drugs and hydroxocobalamin, this patient gradually recovered and showed no recurrence of PAH during follow-up.

Patient 7 was admitted at 6 years of age due to systemic hypertension, haematuria, proteinuria, and anemia to a local hospital, and echocardiography revealed LV hypertrophy, and enlargement, impairment of global LV systolic (EF, 51; FS, 26%), and diastolic function with no signs of PAH, which was probably secondary to the systemic hypertension and anemia. This patient was then transferred to a higher level hospital and diagnosed with the CblC type harboring heterozygous variants (c.80A>G/c.217c>T). Renal biopsy revealed endocapillary proliferative glomerulonephritis. After treatment with antihypertensive drugs, nephroprotective agents and hydroxocobalamin, control of this patient's blood pressure was achieved, with improved cardiac function and negative conversion of haematuria and proteinuria.

Patient 8 first showed narcolepsy, vomiting, jaundice, anemia and dyspnoea at 4 years of age and was then diagnosed with the CblC type harboring variants (c.609G>A/c.80A>G). 1 month later, this child was transferred to the ICU due to the sudden onset of respiratory failure. He presented with hypovolemic shock and decompensated metabolic acidosis, and fibreoptic bronchoscopy with bronchoalveolar lavage indicated alveolar haemorrhagic syndrome (AHS). Echocardiography revealed moderate PAH with a PASP of 62 mmHg and LV enlargement with impaired mobility. In addition, a venous thrombus was detected in the left superficial jugular vein. With aggressive symptomatic treatment and hydroxocobalamin, this patient was rescued.

As mentioned above, patients 6–8 shared a common *MMACHC* variant, c.80A>G. In addition, only patient 6 had mild PAH, while the others showed no signs of PAH at the time of echocardiography.

DISCUSSION

Myocardial Dysfunction

According to our study, the heart was smaller in both MMA subtypes, as the LVIDD and LVMI were lower in the patients than the control subjects, this may play a role in their increased heart rate as higher frequency was required to fulfill their physiological need for circulating a sufficient blood volume.

In terms of systolic function, the LV GLS showed a reduction of 1.4 and 1.1% in isolated MMA and combined MMA group, respectively, indicating impaired systolic function, although the EF was increased in all MMA patients. This inconsistency may be due to the cubed method that presumes the heart as a cube which is not an accurate model. In addition, we have analyzed the relationship of GLS and age in different groups (**Supplementary Figure 2**) by linear regression model. Both isolated and combined MMA groups had a significant lower intercept compared to control group, meanwhile the combined MMA group showed a steeper slope suggesting that this group suffered a more severe adverse effect along with time. And the results of TAPSE suggested that RV systolic function was impaired only in combined MMA patients. As for diastolic function, in combined MMA patients, our study showed a lower E' velocity in both ventricles, while isolated MMA group only exhibited reduced LV E'. Thus we summarized that isolated MMA and combined MMA group showed different pattern of cardiac function impairment, in which the former only affected the left ventricle while the latter affected both ventricles.

Although the pathophysiology of this disease remains unclear, there are several explanatory hypotheses, including direct toxicity of excess metabolites (methylmalonic acid and homocysteine), enhanced oxidative stress and mitochondrial disorders (1, 25–27). For isolated MMA, the pathogenic mechanism may be closer to that of propionic acidaemia, which is mainly related to the disorder of the tricarboxylic acid cycle and the damage of the respiratory chain. Due to direct cytotoxic effects of accumulated propionic acid and methylmalonyl, the level of reactive oxygen species increases dramatically with reduced activity of antioxidant enzymes, causing other side-effects such as lipid peroxidation, protein carbonylation, and oxidation of mitochondrial DNA. In addition, extra-accumulation of mitochondrial permeability transition pore increases the non-selective permeability of the mitochondrial membrane, leading to the loss of reduced coenzymes I and II, calcium ions, which reduces membrane potential, and causes mitochondrial oedema. These processes severely damage the function of mitochondria, and then promote the synthesis of reactive oxygen species, thus forming a vicious circle. On the other hand, the accumulation of methylmalonic acid and propionate competitively consumes CoA to synthesize methylmalonyl-CoA and propionyl-CoA, while the heart tissue lacks the corresponding carnitine acyltransferase and cannot release CoA by displacement reaction. Thus, the tricarboxylic acid cycle is inhibited, resulting in a cardiac metabolism transition from fatty acid oxidation to sugar catabolism, which is similar in patients with heart failure (28, 29).

For combined MMA patients, extra mechanisms relating homocysteine may be involved. Hyperhomocysteinaemia can induce endothelial-myocyte uncoupling by matrix metalloproteinase activation and subsequent interstitial fibrosis accumulation, and this uncoupling leads to impaired diastolic relaxation (30, 31). In addition, beta2-adrenergic receptor was found to be down-regulated due to homocysteine, which contributed to the impaired contractile function of cardiomyocytes in diabetic cardiomyopathy (32).

Higher Prevalence of CHD

In our study, combined MMA patients showed a much higher incidence of CHD of ∼5.3% compared to that of 8.98 per 1,000 live births in the general population in China (33).

In CblC patients, because of insufficient MeCbl, the remethylation of homocysteine (Hcy) to methionine (Met) catalyzed by methionine synthase is greatly impaired, causing disturbance or disruption of the Met-Hcy-SAM pathway. S-adenosylmethionine (SAM) is an important methyl group donor and participates in various methylation reactions, including DNA and histone methylation (34, 35). A growing body of research has shown a strong connection between DNA or histone methylation disorders and the occurrence of CHD. A case-control study conducted by Sylvia et al. (36) pointed out that Down syndrome and CHD may be associated with a global hypomethylation status, as they showed a higher S-adenosylhomocysteine (SAH) level and lower SAM: SAH ratios. Through the analysis of genome-wide DNA methylation data from myocardial biopsies in CHD patients, Marcel et al. (37) found that the aberrant methylation of promoter CpG islands and methylation alterations could result in differences in DNA splicing and contribute to the occurrence of CHD. Apart from DNA methylation, histone methylation modification, as an important epigenetic regulatory component, has been verified to be involved in the development of heart and blood vessels. Variants and deficiencies in histone methylation-modifying enzymes have resulted in various cardiac abnormalities in different species (38). Moreover, the *MMACHC* gene itself has shown tissue-specific expression in the developing heart in mouse embryos, indicating its involvement in cardiac development (39). An *MMACHC* proteomic analysis suggested that CblC variants led to broad metabolic dysfunction, including dysregulation of the cytoskeleton and cell signaling. Pathway analysis demonstrated a strong association with cardiovascular disease, especially cardiomyopathy, due to excessive collagen production (40).

Potential Thrombus-Related Diseases

Another form of cardiovascular involvement is acute heart failure, which is more critical and life-threatening. To date, the associated variants have shown strong heterogeneity among races. In European countries, c.271dupA and c.276G>T were the two leading variants detected in CblC patients with isolated PAH or a combination of PAH and atypical haemolytic uraemic syndrome (aHUS) (6, 10, 41, 42), while in China, c.80A>G was the leading variant detected (8, 9, 43, 44). In a study including 15 MMA/HCY patients with PAH, genetic diagnosis was performed in ten patients, and all of them carried the *MMACHC* c.80A>G variant (43). Our group also collected twelve CblC type patients with PAH, and only one patient did not have the c.80A>G variant (data unpublished). The strong connection of this variant with PAH should be further investigated.

The mechanism was suspected to be hyperhomocysteinaemia-related thrombotic microangiopathy (TMA), as thrombi were detected in pulmonary vessels and on renal biopsy, and homocysteine was recognized as a risk factor for arteriosclerosis and thrombosis in adults. However, there was controversy

as thrombosis was not found in all patients with PAH, and some patients presented with interstitial lung disease or pulmonary vessel abnormalities (9, 43, 44). In our study, patient 8 was diagnosed with AHS and showed no indications of pulmonary embolism. These diverse clinical manifestations suggest the involvement of different mechanisms, including thrombosis, endothelial damage and vascular dysplasia. In addition, pulmonary hypertension was also reported in isolated MMA patients (45, 46), although the incidence was lower; thus, there may be a synergistic mechanism of involving methylmalonic acid and homocysteine in CblC patients leading to this clinical manifestation.

CONCLUSION

Isolated and combined MMA groups showed different pattern of cardiac function impairment, in which the former only affected the left ventricle while the latter affected both ventricles, and affected ventricle exhibited both systolic and diastolic function impairment. In addition, there was a relatively high incidence of CHD in combined MMA group, and we recommended that combined MMA patients should undergo routine cardiovascular examinations. For patients carrying the *MMACHC* c.80A>G variant, extra attention should be paid to signs of PAH.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YW, KS, and LH contributed equally to the study and conceived and designed the study. YL and LY prepared an analytical plan, analyzed data, and drafted the initial manuscript. RS was involved in the clinical data collection. SH and BZ were involved in the electrocardiography. YW performed the echocardiography examinations. All authors have reviewed and revised the manuscript, approved the final manuscript as submitted, and agreed to be accountable for all aspects of the work.

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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.2022.810495/full#supplementary-material

Supplementary Figure 1 | Enrolment of MMA patients. 99 MMA patients enrolled, including 23 isolated MMA patients and 76 combined MMA patients. Isolated MMA patients consisted of 18 patients caused by methylmalonyl-CoA mutase (MUT) variant (OMIM# 251000), 1 MMAA variant (OMIM# 251100), and 1 MMAB variant (OMIM# 251110). 64 combined MMA patients had identified *MMACHC* variant (OMIM #277400).

Supplementary Figure 2 | Effect of age on the GLS in control, isolated MMA and combined MMA groups.

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Complement C1q Binding Protein (C1QBP): Physiological Functions, Mutation-Associated Mitochondrial Cardiomyopathy and Current Disease Models

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Complement C1q binding protein (C1QBP, p32) is primarily localized in mitochondrial matrix and associated with mitochondrial oxidative phosphorylative function. C1QBP deficiency presents as a mitochondrial disorder involving multiple organ systems. Recently, disease associated C1QBP mutations have been identified in patients with a combined oxidative phosphorylation deficiency taking an autosomal recessive inherited pattern. The clinical spectrum ranges from intrauterine growth restriction to childhood (cardio) myopathy and late-onset progressive external ophthalmoplegia. This review summarizes the physiological functions of C1QBP, its mutation-associated mitochondrial cardiomyopathy shown in the reported available patients and current experimental disease platforms modeling these conditions.

Keywords: C1QPB, mutation, combined oxidative phosphorylation deficiency, mitochondrial cardiomyopathies, physiological functions, disease models

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INTRODUCTION

Mitochondrial diseases form a diverse group of heritable disorders caused by a wide spectrum of mutations in nuclear or mitochondrial genes (1, 2). The nuclear DNA encodes over 1,000 mitochondrially localizing proteins, translated in the cytoplasm, and translocated to the mitochondria. The mitochondrial (mt-)DNA encodes 37 genes including 22 tRNAs and two rRNAs. The mt-DNA is essential for mt-DNA-specific translation of the 13 encoded respiratory chain subunits (3). Dysfunction in mitochondrial respiratory chain function and cellular energy production affects different tissues, owing to critical dependence of the heart on oxidative metabolism; cardiac involvement in mitochondrial disease is common and may occur as the principal clinical manifestation or part of multisystem disease (4, 5). Cardiovascular involvement in mitochondrial disease resulted in lower survival rates to age 16 years than in patients without heart disease (6).

C1QBP comprises 6 exons located on the short arm of chromosome 17p13.2. It is highly conserved through evolution (7): the human and rat/mouse cDNA sequences are almost identical (8, 9). *C1QBP* encodes the complement component 1Q subcomponent-binding protein (C1QBP, p32). This widely expressed multifunctional protein

is predominantly localized in mitochondrial matrix (10, 11). Its N-terminal mitochondrial targeting peptide is proteolytically processed after it reaches the mitochondrial matrix. Here it forms a doughnut-shaped homotrimeric complex (12–14).

C1QBP is reported to exert pleiotropic effects on many cellular processes, including mitochondrial homeostasis, mitochondrial oxidative phosphorylation (OXPHOS) and in nucleus–mitochondrial interactions, inflammation, and cancer (15–19). Biallelic *C1QBP* mutations were recently identified manifesting as combined oxidative phosphorylation deficiency (COXPD) in an autosomal recessive inherited pattern. This involved multiple systems including heart, liver, skeletal muscle, eye and nervous system (20–24). However, cardiomyopathy, whose exact underlying mechanisms remain elusive, is the major phenotype. This review summarizes physiological functions of C1QBP, its mutation-associated mitochondrial cardiomyopathy, and current disease models.

PHYSIOLOGICAL FUNCTIONS OF C1QBP

C1QBP is highly expressed in cells with substantial energy metabolism such as cardiac and skeletal muscle (25). It can exist in numerous cellular compartments but is predominantly targeted to the mitochondria reflecting the mitochondrial targeting sequence in its 73N-terminal amino acids. The C1QBP crystal structure reveals three monomers forming a doughnut-shaped quaternary structure with an unusually asymmetric surface charge distribution. It shows a fold comprising seven consecutive antiparallel β-strands flanked by one N-terminal and two C-terminal α-helices (25–29).

Mitochondrial C1QBP is essential for OXPHOS. It pivotally supports translation of the mitochondrially encoded respiratory chain protein complexes I, III, IV and V (30). C1QBP knockdown decreased complexes I, III, IV, and V but not complex II levels, reducing complex I and IV enzymatic activity (16). Chen et al. reported that C1QBP binds to the core, dihydrolipoyl-lysine-residue acetyltransferase (DLAT), component of the matrix multienzyme pyruvate dehydrogenase (PDH) complex (31). PDH is important in energy homeostasis, linking glycolysis and the tricarboxylic acid (TCA) cycle. PDH catalyzes pyruvate conversion to acetyl-CoA used in the TCA cycle to generate ATP (32). C1QBP regulates OXPHOS through binding to DLAT, providing a novel molecular mechanism by which C1QBP regulates cellular respiration (27).

C1QBP is required for development and maintenance of normal cardiac function (15). Cardiomyocyte specific C1QBP deletion resulted in contractile dysfunction, cardiac dilatation and fibrosis. C1QBP deficiency decreases COX1 expression and oxygen consumption rates, increased oxidative stress, further leading to cardiomyocyte dysfunction. In cardiomyocytes, C1QBP acts as an RNA and protein chaperone modulating mitochondrial translation and function (15). In addition, 5' adenosine monophosphate-activated protein kinase A (AMPKA) was constitutively phosphorylated, and eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) and ribosomal S6 kinase (S6K) were less phosphorylated in C1QBP deficient myocytes, suggesting impaired rapamycin signaling. Metabolic analysis also demonstrated an impaired urea cycle in C1QBP deficient hearts (15).

Mitochondrial morphology is closely linked to energy metabolism. Reduced OXPHOS and enhanced glycolysis correlates with mitochondria fragmentation and mitochondrial matrix expansion (33). C1QBP is required to maintain normal mitochondrial structure and is critical in protecting mitochondria from fragmentation and swelling by inhibiting OMA1-dependent proteolytic processing of the optic atrophy type 1 GTPase protein (OPA1) (15, 28). Cardiac mitochondria from C1QBP-deficient mice showed disordered alignment, enlargement and abnormalities in their internal structure (15). Furthermore, the mitochondrial network was fragmented rather than fibrillar when C1QBP was not expressed (16).

The crystal structure of C1QBP has been determined. This is compatible with an association with regulation of mitochondrial Ca^{2+}. It can form a pore-like homotrimer that could serve as a high-capacity divalent cation storage protein. C1QBP contains 282 amino acid residues. Twenty three percent of these residues are glutamic and aspartic acids distributed on the trimer surface. This characteristic acidic surface is reminiscent of the major sarcoplasmic reticular Ca^{2+} storage protein calsequestrin (34). The latter modulates intracellular Ca^{2+} concentration and affects membrane Ca^{2+} transport rates into sarcoplasmic reticular vesicles. C1QBP may similarly modulate Ca^{2+} levels in the mitochondrial matrix (14). Xiao et al. proposed that C1QBP protein is additionally a positive regulator of mitochondrial Ca^{2+} uptake (35). Koo et al. and Choi et al. similarly confirmed that mitochondrial C1QBP protein has a regulatory effect on mitochondrial Ca^{2+} uptake (36–38). Oxidative metabolism strongly varies with mitochondrial Ca^{2+} levels (39). PDH, NAD-isocitrate dehydrogenase and oxoglutarate dehydrogenase are all regulated by intramitochondrial Ca^{2+} levels either directly or indirectly (40). C1QBP may thereby regulate mitochondrial OXPHOS by modulating Ca^{2+} concentrations (14).

C1QBP expression increases in multiple cancer cells in human, including breast, endometrial, ovarian, prostate, melanoma, lung, and colon cancer (41–49). C1QBP may be pivotal in tumor cell survival, growth and metastatic invasion through interacting with critical molecules, including those of the complement and kinin systems, in the tumor cell microenvironment (25). C1QBP may be needed to sustain tumor cell growth by maintaining respiration and OXPHOS. C1QBP knockdown tumor cell lines showed decreased complex I, III, IV, and V subunit levels (50). Zhang et al. (51) suggested that C1QBP further regulated protein kinase Cζ activity and modulated EGF-induced cancer cell chemotaxis. It was additionally identified as a novel regulator of cancer metastasis that may serve as a target for breast cancer therapy (50).

Finally C1QBP appears critical in inflammation processes and responses to infection (25). It binds to a wide variety of plasma and cell surface, and pathogenic microorganism proteins. It is critical in modulating fibrin formation, particularly at local sites of immune injury and/or inflammation and activating the kinin/kallikrein system. It is also able to generate the powerful

TABLE 1 | Disease-associated mutations of C1QBP in mitochondrial disease.

Case No.	Site of mutation	Type of mutation	Location		mtDNA damage	MRC complex activities	Ethnicity	Gender	Age of onset	Outcome	Involved system	Symptoms		References
			exons	protein								Cadiovasular system	Other system	
1	c. 557G>C; p. Cys186Ser; c. 612C>G; p. Phe204Leu	compound heterozygous mutations	4; 5	β strand; coiled-coil region	Copy number variation	Muscle: I/CS: 27% II/CS: 64% III/CS: 8% IV/CS: 82%	British	male	4 days	deceased (18 days)	heart; CNS; kidney	cardiorespiratory arrest, asymmetric left ventricular cardiomegaly	multiple cortical, ventricular, and subdural hemorrhages and cerebral edema, burst suppression-like electrical discharges, subclinical seizures, congenital nephrosis, hypothyroidism, disseminated intravascular coagulopathy	Feichtinger et al. (20)
2	c.739G>T; p. Gly247Trp; c.824T>C p. Leu275Pro	compound heterozygous mutations	6	hydrogen-bonded turn; αC helix	Copy number variation	Liver: I/CS: 6% II/CS: 36% III/CS: 22% IV/CS: 13%	Japanese	female	birth	deceased (4 days)	heart; liver	cardiomegaly	hepatomegaly	Feichtinger et al. (20)
3	c. 823C>T; p. Leu275Phe	homozygous mutations	6	αC helix	mtDNA deletions	Muscle: I/CS: 12% I+III/CS: 63% III/CS: 8% IV/CS: 6%	Austrian	male	5 years	alive (22 years)	heart; liver; PNS; muscle; eye	left ventricular hypertrophy	increased transaminases; sensory peripheral neuropathy, exercise intolerance with fatigue and vomiting, astigmatism, amblyopia, ptosis, PEO	Feichtinger et al. (20)
4	c.562_564del; p. Tyr188del	homozygous mutations	4	coiled-coil region	mtDNA deletions	Muscle: I/CS: 55% I+III/CS: 52% II/CS: 57% II+III/CS: 33% IV/CS: 46%	Italian	male	57 years	deceased (70 years)	heart; PNS; muscle; eye	left ventricular hypertrophy	diffuse neurogenic abnormalities and focal myogenic in the gluteus maximus, exercise intolerance, weakness, ptosis, PEO, post-traumatic depression, diabetes, sensorineural hearing loss	Feichtinger et al. (20)
5	c.612C>G p.Phe204Leu	homozygous mutations	5	coiled-coil region	mtDNA deletions	Muscle: I/CS: about 40% II/CS: about 140% III/CS: about 60% IV/CS: about 40%	Italian	female	28 years	alive (54 years)	eye; muscle;	Nil	PEO, bilateral ptosis, almost complete ophthalmoparesis, severe dysphagia, and rhinolalia	Marchet et al. (21)
6	c.562_564del p.Tyr188del	homozygous mutations	4	coiled-coil region	mtDNA deletions	Muscle: I/CS: about 80% II/CS: about 180% III/CS: about 100% IV/CS: about 100%	Italian	female	30 years	alive (65 years)	eye; muscle; PNS;	Nil	PEO; bilateral ptosis, hyposthenia, swallowing dysfunction, decreased exercise tolerance dysfunctions in executive and visuospatial areas	Marchet et al. (21)
7	c. 823C>T p. Leu275Phe	homozygous mutations	6	αC helix	Nil	NA	Chinese	male	1.5 years	alive (14 years)	heart; muscle; eye	left ventricular hypertrophy	decreased exercise tolerance; ptosis	Wang et al. (22)

(Continued)

TABLE 1 | Continued

Case No.	Site of mutation	Type of mutation	Location		mtDNA damage	MRC complex activities	Ethnicity	Gender	Age of onset	Outcome	Involved system	Symptoms		References
			exons	protein								Cadiovasular system	Other system	
8	c. 823C>T p. Leu275Phe	homozygous mutations	6	αC helix	Nil	NA	Chinese	male	2 years	alive (9 years)	heart; muscle; eye	left ventricular hypertrophy	decreased exercise tolerance; ptosis	Wang et al. (22)
9	c.743T>C p.Val248Ala	homozygous mutations	6	hydrogen-bonded turn	NA	NA	Syrian	male	fetuses	deceased (33 weeks gestational age)	heart; liver;	cardiomyopathy	IUGR, oligo/anhydramnios, generalized edema, cardio/hepatomegaly, cortical hemorrhages, and preterm birth	Alstrup et al. (23)
10	c.743T>C p.Val248Ala	homozygous mutations	6	hydrogen-bonded turn	NA	Fibroblasts: II: 0.57 (reference range, 0.38–0.76) III: 1.6 (reference range, 1.0–1.8) IV: 0.7 (reference range, 1.2–3.2)	Syrian	female	fetuses	deceased (20 weeks gestational age)	heart; liver;	cardiomyopathy	IUGR, oligo/anhydramnios, generalized edema, cardio/hepatomegaly, cortical hemorrhages, and preterm birth	Alstrup et al. (23)
11	c.118dupA; p.Thr40Asnfs *45; c.612C>G; p.Phe204Leu	compound heterozygous mutations	1	truncation; coiled-coil region	NA	NA	NA	female	7 months	deceased (7 months)	heart	left ventricular hypertrophy, cardiac failure, ventricular arrhythmias	NA	Webster et al. (24)
12	c.118dupA; p.Thr40Asnfs *45; c.612C>G; p.Phe204Leu	compound heterozygous mutations	1	truncation; coiled-coil region	NA	NA	NA	male	birth	deceased (27 days later)	heart	left ventricular hypertrophy, cardiac failure; ventricular arrhythmias	NA	Webster et al. (23)

CNS, central nervous system; CS, citrate synthase; NA, not available; MRC, mitochondrial respiratory chain; PEO, progressive external ophthalmoplegia; PNS, peripheral nervous system; IUGR, intrauterine growth restriction.

vasoactive peptide bradykinin largely responsible for the swelling seen in angioedema (17–19).

C1QBP MUTATIONS AND HUMAN MITOCHONDRIAL CARDIOMYOPATHY

Biallelic C1QBP mutations were first reported in four individuals by Feichtinger et al. (20). Biallelic C1QBP mutation caused a COXPD 33 (OMIM:617713). In the reported 12 cases with C1QBP mutations, phenotypes were typically severe, even fatal (20–24). They present at any age and cover a wide spectrum of clinical manifestations including intrauterine growth restriction, cardiorespiratory arrest, cardiac hypertrophy, cardiac failure, ventricular arrhythmias, hepatomegaly, exercise intolerance, progressive external ophthalmoplegia (PEO), cerebral hemorrhages/edema and nervous system dysfunction.

Mutations in C1QBP and Clinical Characteristics

Amongst the 12 reported patients there were eight C1QBP amino acid changes, summarized in **Table 1**. **Figure 1** illustrates the gene (**Figure 1A**) and protein structures (**Figures 1B,C**) indicating the positions of C1QBP variants. **Table 2** summarizes the relative frequency of symptoms associated with biallelic variants in C1QBP and the relates the Human Phenotype

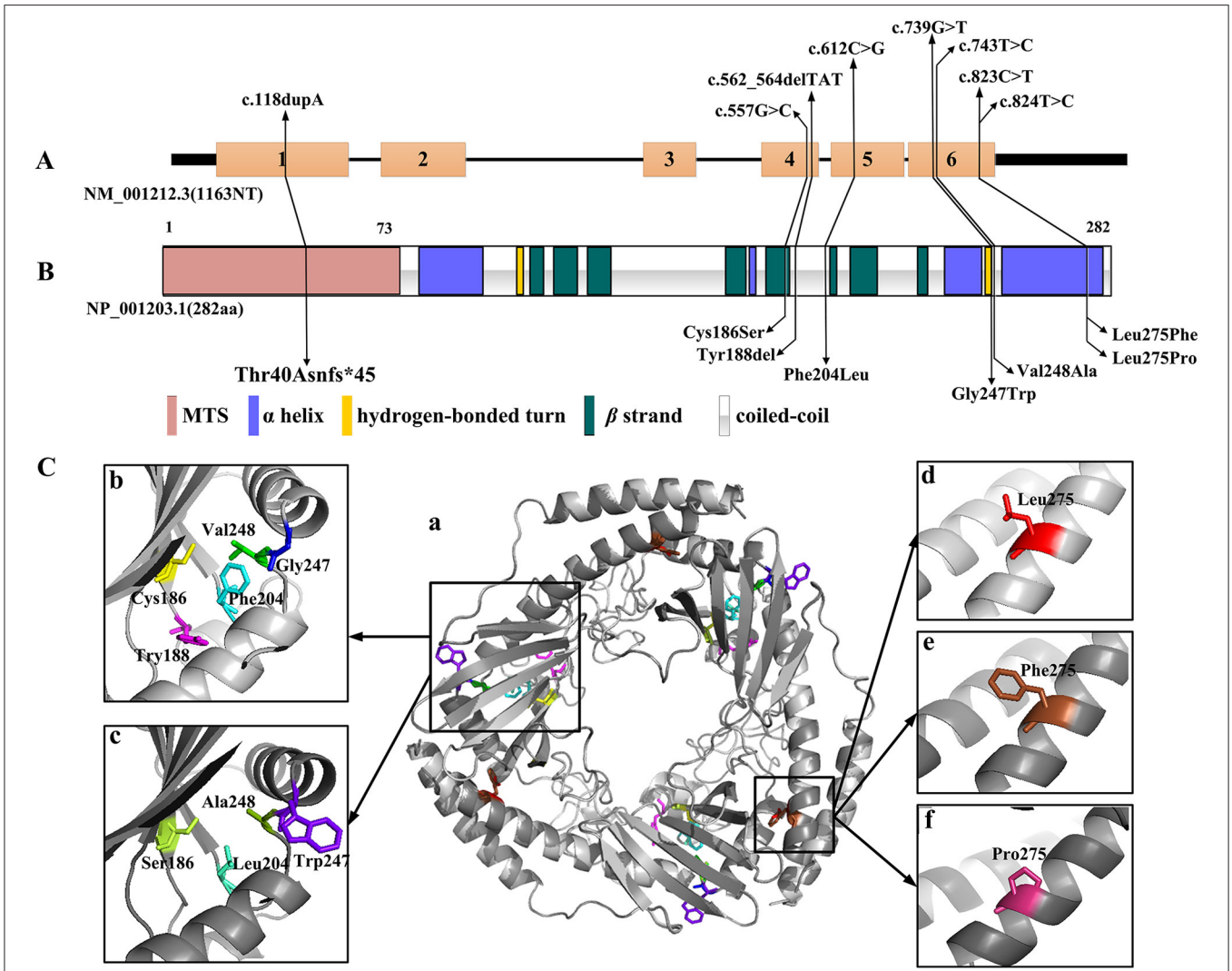

FIGURE 1 | C1QBP variants in gene and protein structure. **(A)** Gene structure with exons and introns showing the localization of the variants. **(B)** Secondary structure of the C1QBP indicating the positions of the variants. MTS indicates the mitochondrial target sequence. **(C)** Inspection of the C1QBP structure performed using PyMOL (PDB accession codes 1P32, https://www.rcsb.org/structure/1P32). a: Predicted three-dimensional structure of the C1QBP protein; b and d: wild type; c, e and f: mutation type. Residue changes are colored in the structure: Cys186, yellow; Ser186, lemon; Tyr188, magenta; Phe204, cyan; Leu204, green-cyan; Gly247, blue; Trp247, purple-blue; Val248, green; Ala248, forest; Leu275, red; Phe275, brown; Pro275, warm pink.

TABLE 2 | The relative frequency of symptoms associated with biallelic variants in C1QBP and the related HPO terms.

HPO terms	Frequency	C1QBP mutations
Autosomal recessive inheritance	12/12	(p. Cys186Ser; p. Phe204Leu); (p. Gly247Trp; p. Leu275Pro); (p. Leu275Phe); (p. Tyr188del); (p. Phe204Leu); (p.Val248Ala); (p.Phe204Leu; p.Thr40Asnfs*45)
Cardiomyopathy	10/12	(p. Cys186Ser; p. Phe204Leu); (p. Gly247Trp; p. Leu275Pro); (p. Leu275Phe); (p. Tyr188del); (p.Val248Ala); (p.Phe204Leu; p.Thr40Asnfs*45)
Left ventricular hypertrophy	7/12	(p. Cys186Ser; p. Phe204Leu); (p. Leu275Phe); (p. Tyr188del) (p.Phe204Leu; p.Thr40Asnfs*45)
Ptosis	6/12	(p. Leu275Phe); (p. Tyr188del); (p. Phe204Leu)
Exercise tolerance	5/12	(p. Leu275Phe); (p. Tyr188del)
Progressive external ophthalmoplegia	5/12	(p. Leu275Phe); (p. Tyr188del); (p. Phe204Leu)
Cardiomegaly	4/12	(p. Cys186Ser; p. Phe204Leu); (p. Gly247Trp; p. Leu275Pro); (p.Val248Ala)
Hepatomegaly	3/12	(p. Gly247Trp; p. Leu275Pro); (p.Val248Ala)
Astigmatism	2/12	(p. Leu275Phe)
Generalized edema	2/12	(p.Val248Ala)
Ventricular arrhythmias	2/12	(p.Phe204Leu; p.Thr40Asnfs*45)
Amblyopia	1/12	(p. Leu275Phe)
Cardiorespiratory arrest	1/12	(p. Cys186Ser; p. Phe204Leu);
Cerebral edema	1/12	(p. Cys186Ser; p. Phe204Leu);
Dysphagia	1/12	(p. Phe204Leu)
Fatigue	1/12	(p. Leu275Phe)
Hypothyroidism	1/12	(p. Cys186Ser; p. Phe204Leu)
Nephrotic syndrome	1/12	(p. Cys186Ser; p. Phe204Leu);
Sensory neuropathy	1/12	(p. Leu275Phe)
Sensorineural hearing impairment	1/12	(p. Tyr188del)
Seizures	1/12	(p. Cys186Ser; p. Phe204Leu);
Vomiting	1/12	(p. Leu275Phe)

HPO, Human Phenotype Ontology.
Variants in () refer to mutation. Two sites indicating compound heterozygous mutations, one site indicating homozygous mutations.

Ontology (HPO) terms in which C1QBP mutatins should be suspected when a patient presents with cardiomyopathy, especially left ventricular hypertrophy, cardiomegaly, exercise tolerance, ptosis and PEO.

The p.Phe204Leu mutation of the C1QBP protein was identified in four patients either in homozygosity (case 5) (21) or in compound heterozygosity (case 1, 11, 12) (20, 24). The homozygous p.Phe204Leu mutation described in case 5 showed an adult onset mild phenotype, with PEO and mitochondrial myopathy. The patient is alive without cardiac phenotypes (21). A further three patients with a p.Phe204Leu mutation were identified with a second mutation. The compound heterozygous p.Phe204Leu and p.Cys186Ser mutation occurred in case 1, a newborn baby with cardiorespiratory arrest, asymmetric left ventricular cardiomegaly, multiple cortical, ventricular, and subdural hemorrhages, cerebral edema and burst suppression-like electrical discharges with subclinical seizures (20). The heterozygous frameshift c.118dupA insertion can result in a truncation mutation, p.Thr40Asnfs*45, in the protein (23). The compound heterozygous p.Thr40Asnfs*45 and p.Phe204Leu mutations were noted in two siblings (case 11 and 12) with ventricular arrhythmias, cardiac hypertrophy and cardiac arrest.

The homozygous mutation of p.Leu275Phe was identified in cases 3, 7 and 8. These three patients are all alive and show clinical phenotypes of left ventricular hypertrophy, exercise intolerance and ptosis (20, 22). The homozygous p.Val248Ala mutations were described in case 9 and case 10 (23). They are associated with severe intrauterine growth restriction, edema, and cardiomyopathy. The compound heterozygous p.Gly247Trp and p.Leu275Pro mutations were identified in case 2 who suffered with cardiomegaly and hepatomegaly. She was deceased 4 days after birth. The homozygous p.Tyr188del mutation was identified in case 4 and was associated with left ventricular hypertrophy, exercise intolerance, weakness, ptosis, PEO and peripheral nervous system abnormalities (20). However, this mutation in Marchetet's report was identified with the phenomenon of PEO and mitochondrial myopathy, without cardiac involvement (21).

Genotype-Phenotype Correlation

Among the 12 patients, cases 4, 5 and 6 showed later onsets and longer survivals compared with the remaining patients (20, 21). Their corresponding sites of amino acid change of p.Phe204Leu and p.Tyr188del all fell in the C1QBP protein coiled-coil region. The p.Cys186Ser, p.Gly247Trp, p.Val248Ala, p.Thr40Asnfs*45, p.Leu275Phe and p.Leu275Pro variants associated with early onset cardiomyopathy all occurred in important structural domains, such as the β strand, hydrogen bonded turn, and the αC helix. In particular, the p.Leu275Phe mutation is located in the αC helix, and three patients carrying this mutation all remain alive despite early onset combined OXPHOS deficiency and cardiac hypertrophy. These observations suggest that the localization of the pathogenic variants within the C1QBP protein structure show correlations with the various observed phenotypes.

C1QBP Variant Protein Structure Predictions

The three-dimensional (3D) C1QBP structure was analyzed using the wild type protein (PDB accession codes 1P32, https://www.rcsb.org/structure/1P32) and the SWISS-MODEL (http://swissmodel.expasy.org/). **Figure 1C** was acquired with the PyMOL molecular graphics system (PyMOL, https://pymol.org/2/). The truncation mutation of p.Thr40Asnfs*45, located in the N-terminal mitochondrial targeting peptide proteolytically processed after C1QBP import into the mitochondrion, was not present in the 3D structure. Amino acid changes have

pivotal effects on protein structure and hydrophobic surface exposure, especially the polarity and hydrophilic/hydrophobic differences after mutation. Cys186 and Ser186 are neutral amino acids. Val248 and Ala248 are nonpolar amino acids with hydrophobic side chains. Phe 204, Phe275 and Pro 275 have aromatic amino acid side chains, Leu204, Leu275 with fatty acid side chain, are all nonpolar. Thus the mutations of Cys186Ser, Val248Ala, Phe204Leu, Leu275Phe and Leu275Pro may not significantly alter the protein structure. Tyr is the aromatic amino acid with hydrophobic side chains; thus the Tyr188del mutation may result in a decreased exposure of the hydrophobic surface. Gly247 and Try247 have different polarity and side chains, suggesting that that the Gly247Tyr variant may increase the exposure of the hydrophobic surface. However, perspectives from structural change alone may not be sufficient to analyse the functional alterations without further verification.

MtDNA Damage

The mtDNA damage is often associated with PEO (52). mtDNA copy number variants and multiple mtDNA deletions were identified in cases 1-8. Case 1 (p.Cys186Ser; p.Phe204Leu) and case 2 (p.Gly247Trp; p.Leu275Pro) showed higher mtDNA copy numbers in muscle and liver samples, but there was no evidence of mtDNA rearrangements (20). Case 3 (p.Leu275Phe) and case 4 (p.Tyr188del) showed multiple mtDNA deletions in muscle samples; both have a PEO phenotype (20). Similarly, Marchet et al. (21) reported two unrelated adult patients, presenting with PEO; muscle biopsies from both carried multiple mtDNA deletions. Our group reported a homozygous C1QBP-Leu275Phe mutation in case 7 and case 8 with ptosis instead of PEO, and no mtDNA deletion was detected in the blood samples of both patients and their parents (22). Nevertheless, determining whether PEO is linked to C1QBP mutation and mtDNA damage needs an expansion of the cohort of patients carrying C1QBP.

Physiological Consequences

Muscle biopsies were obtained and described in cases 1–6 and cases 9 and 10. Feichtinger et al. (20) described four individuals, all with cardiac symptoms. Their respiratory chain activities in muscle or liver homogenates showed severe deficiency. Muscle homogenates from proband case 1, case 3, and case 4 showed decreased complex I and complex IV subunits, consistent with the findings of enzymatic investigations from muscle (20). Furthermore, case 3 and case 4 showed increased mitochondrial mass indices. Marchet et al. reported two unrelated adult patients from consanguineous families, presenting with PEO, mitochondrial myopathy, without cardiac involvement. Muscle biopsies from both patients showed the typical mitochondrial alterations. Spectrophotometric analysis of the mitochondrial respiratory chain complexes in muscle homogenates showed partially reduced complex I, III, and IV activities in case 5, whereas values were in the control range for case 6 (21). Alstrupet et al. reported that the analysis of a fibroblast culture from one of the fetuses showed a deficiency of respiratory chain complex IV (23). The muscle biopsy analysis indicates that the degree of respiratory chain complex deficiency may correlate with phenotype and genotype.

MODELS OF MITOCHONDRIAL DISEASE ASSOCIATED WITH C1QBP MUTATIONS

Animal Model

Toshiro et al. generated cardiomyocyte-specific conditional C1QBP knockout (cKO) mice using the Cre-loxP approach (15). C1QBP-deficient mouse hearts showed altered mitochondrial structure and function corresponding to an increased oxidative stress, further leading to cardiomyocyte dysfunction. Furthermore, C1QBP-cKO mice presented with embryonic lethality with the embryonic fibroblasts showing multiple OXPHOS defects. Oxygen consumption rates, and mitochondrial and cytosolic translation were inhibited in C1QBP-cKO mice. C1QBP-deficient hearts also showed increased ornithine and decreased citrulline metabolites, suggesting that the urea cycle was affected. In conclusion, mitochondrial dysfunction caused by C1QBP deficiency affects cellular homeostasis and induces a protective response against cardiomyopathy (15).

IPSC-CMs

Development of human induced pluripotent stem cells (hiPSC) has initiated a new era of *in vitro* cell model reconstruction and research into individual pathogenesis of mutation-specific diseases in patients. Somatic cells after reprogramming carry all genetic information including pathogenic genes. Cardiomyocytes differentiated from iPSCs (iPSC-CMs) can reproduce disease phenotypes, thus providing an important platform for studying pathogenesis (53). Our group has established iPSCs carrying the C1QBP-L275F mutation (54). The C1QBP-L275F-iPSC-CMs showed a cardiomyocyte hypertrophy phenotype in common with our patient. The cross-sectional area of iPSC-CMs derived from the proband was significantly increased compared to the mothers'. The C1QBP protein was distributed in the mitochondria. Electron microscopy showed that these were disordered in their morphology, number and size. Therefore, this is likely to become a successful model to provide a pivotal platform for studying the pathogenesis of mitochondrial cardiomyopathy caused by C1QBP-L275F mutations.

SUMMARY

We summarize the structure and physiological functions of C1QBP and its mutation related clinical phenotypes. C1QBP localizes predominantly in the mitochondrial matrix and is essential for OXPHOS maintenance. Patients identified with C1QBP mutations showed combined respiratory-chain deficiencies and abnormalities in the heart, liver, kidney, skeletal muscle, eye and nervous system. Clinical manifestations included intrauterine growth restriction, cardiomyopathy, hepatomegaly, exercise intolerance, swallowing dysfunction, ptosis, PEO and peripheral nervous system dysfunction. The relationship between observed mitochondrial cardiomyopathy and the C1QBP mutations and its underlying mechanism

requires further studies for its elucidation on the platforms of iPSC-CMs and animal models.

AUTHOR CONTRIBUTIONS

YZ wrote the original manuscript. YZ and CL-HH reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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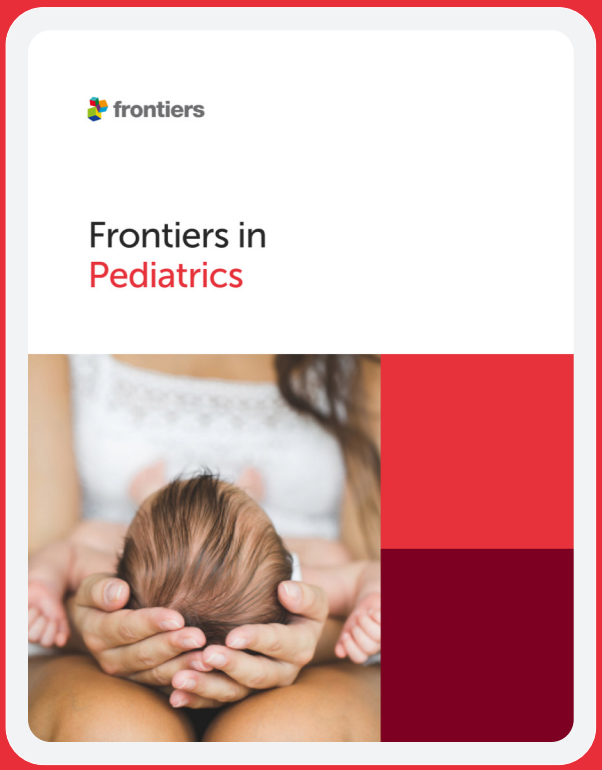

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