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RESEARCH

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Genetic testing and family screening in idiopathic pediatric cardiomyopathy: a prospective observational study from a tertiary care center in North India

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Abstract

Introduction There are limited data on family screening and genetic testing in pediatric cardiomyopathy from India. This study was conducted to describe the morphologic spectrum and identify potential familial and genetic causes of pediatric cardiomyopathies in this region.

Methods From April 2018 to May 2020, all children from birth to 18 years of age with cardiomyopathy visiting a tertiary care hospital in North India were enrolled in this study. First-degree relatives of index patients were offered screening for cardiomyopathy; 260 clinically reported pathogenic/likely pathogenic variants in 17 genes were analyzed by a rapid genotyping method. Additionally, a subset of patients also underwent whole-exome sequencing.

Results Of the 20 patients enrolled in this study (median age 42 months), 18 were clinically diagnosed with dilated cardiomyopathy. We observed a 44.4% mortality rate after a median follow-up of 15 months. 61.3% of the eligible first-degree relatives underwent screening, and one patient was identified to have familial cardiomyopathy. Multi-panel gene testing was performed on 18 patients, and none were found to have a pathogenic or likely pathogenic variant; 9 patients also underwent whole-exome sequencing, and pathogenic and likely pathogenic variants were identified in 50% (4/8) of them.

Conclusion Dilated cardiomyopathy is the most common morphologic form of pediatric cardiomyopathy in India and has a high mortality rate. The prevalence of familial cardiomyopathy was low in this study. Future studies should evaluate the role of whole-exome sequencing in identifying genetic causes of cardiomyopathy in children.

Keywords Cardiomyopathy, Pediatrics, Genetics, Whole-exome sequencing

Introduction

Pediatric cardiomyopathies are a group of heterogeneous disorders of diverse etiologies, subdivided into morphological subtypes like dilated, hypertrophic, restrictive, etc. Just like their etiologies, their course and prognosis are also varied [1–3]. Increased understanding of the genetic factors driving the pathogenesis of cardiomyopathies and the improvement in genetic testing has led to a more efficient and rapid diagnosis. Genetic screening could lead to a better prognosis, improving the overall

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effectiveness of treatment and chances of survival. Family history assessment and screening of family members are imperative in identifying genetic and familial cases of cardiomyopathies [4].

The genetic underlying of any heritable disease varies widely across populations, despite similar phenotypic profiles. Only a handful of studies from India have reported on genetic testing in patients with pediatric cardiomyopathy, indicating a severe lack of understanding of the genetic factors driving the disorder in the Indian population [5, 6]. This study was conducted to describe the morphologic spectrum and short-term outcome of pediatric cardiomyopathies in a tertiary care hospital in North India and to identify potential genetic and familial causes.

Material and methods

This study was conducted at the Department of Pediatrics of a tertiary care hospital in North India from April 2018 to May 2020. The study was done under the institutional ethics committee approval No.196(15/2017)/IEC/PGIMER/RMLH454/18 and No. IGIB/IHEC/GOMED/2022-2023. Patients were enrolled once they fulfilled the inclusion and exclusion criteria after taking informed consent; assent was also taken from children more than 7 years of age.

Inclusion criteria

Children from birth to 18 years of age diagnosed with cardiomyopathy.

Exclusion criteria

HIV infection, myocarditis, primary valvular heart diseases, Kawasaki disease, coronary artery disease, hypertension, renal disease, Takayasu arteritis, or other immunologic diseases. Known causes of heart muscle disease (such as anthracyclines and iron overload). Invasive cardiac procedures or cardiac surgery in the past. Rhythm disorders causing cardiomyopathy. Severe chronic anemia, thyroid disorder. Infants of a diabetic mother, storage disorders, inborn errors of metabolism, neuromuscular disorder, dysmorphism, or syndromic diseases.

Clinical workup

Enrolled patients underwent a detailed history and physical examination. Emphasis was given to a history suggestive of viral infection before the onset of cardiac symptoms. Elaborate birth and developmental history, as well as three-generation family history, was taken to construct a pedigree chart. Family history of cardiomyopathy or history of sudden cardiac death in the family was specifically taken into account. Heart failure was scored according to the modified Ross score [7]. Treatment history was recorded and the presence of dysmorphism, organomegaly, or neuromuscular disorder was assessed. Routine investigations included a complete hemogram, renal and liver function tests, electrocardiogram, and chest X-ray. Depending upon the clinical situation HIV tests, other viral serologies, thyroid function tests, and calcium and vitamin D level tests were also performed. If there was a clinical suspicion of the metabolic cause of cardiomyopathy, tandem mass spectrometry (TMS) or gas chromatography mass spectroscopy (GCMS) was performed in selected cases. Echocardiography was conducted using a Philips HD11XE machine following the Guidelines of the American Society of Echocardiography on chamber quantification [8]. Measured echocardiographic parameters include left ventricular end-systolic/diastolic dimensions, maximum left ventricular posterior wall thickness, and interventricular septal thickness. Left ventricular ejection fraction was measured using biplane Simpson's method. Indices of left ventricular diastolic function included mitral peak early (E) and late (A) diastolic flow velocity and peak early and late (medial) mitral diastolic annular velocity by tissue Doppler (e', a'). Patients were classified into different morphologic subtypes according to the echocardiography as follows:

Dilated cardiomyopathy (DCM): left ventricular end-diastolic dimension on echocardiography > 2 standard deviations above the normal with an ejection fraction < 45% (z score > 2 and z-score defined as the number of SD from the body surface area adjusted mean in the normal population). Hypertrophic cardiomyopathy (HCM): left ventricular posterior wall thickness at end-diastole > 2 standard deviations above the normal mean for the body surface area (z score > 2). Restrictive cardiomyopathy (RCM): one or both atria enlarged relative to the ventricles of normal or small size with evidence of impaired diastolic filling and in the absence of marked valvular heart disease. LV non-compaction: highly trabeculated spongiform left ventricle myocardium with multiple interstices. We referred to the Z score published by Pettersen et al. from Detroit [9]. Patients were followed up in the pediatric cardiology clinic till the end of the study or till the time of their death.

Family screening

All first-degree relatives of the index patients were offered screening which included history, physical examination, electrocardiogram, and echocardiography. Familial cardiomyopathy was defined as the presence of one or more first-degree relatives with cardiomyopathy or sudden cardiac death or the presence of pathogenic variants.

Genetic tests

After pretest counselling, a blood sample for genetic testing was taken from the index patient and his/her parents; 5 ml of venous blood was taken by venipuncture. As a preliminary workup, genetic testing was performed using a genome-wide genotyping method, the Infinium Global Screening Array-24 Illumina v2.0 chip (https://emea.support.illumina.com/downloads/infinium-global-screening-array-v2-0-product-files.html), and data visualization was performed on Genome Studio 1.0. Rapid genotyping techniques parallelly screen a large number of pathogenic and likely pathogenic markers linked to multiple genetic disorders. Using the ClinVar Database as a reference, 260 clinically reported pathogenic/likely pathogenic variants in *TNNT2, ACTN2, TNN, MYL3, PLN, PRKAG2, MYPN, VCL, LDB3, MYBPC3, MYL2, MYH7, ACTC1, TPM1, TCAP, TNNI3* and *MYLK2* genes (present in GSA v2.0) for cardiomyopathy were selected for screening. Mutation-positive genes were defined by the presence of a disease-causing variant or likely disease-causing variant, whereas those with variants of unknown significance (VUS) were considered mutation-negative. A subset of patients with DCM also underwent whole-exome sequencing (WES). The selection of patients for WES was not pre-determined and was made depending on the availability of kits at the time of testing. Whole-exome sequencing (WES) was performed using TruSeq Exome Kit (Illumina) for library preparation per the manufacturer's protocol, and sequencing was carried out on Illumina NovaSeq 6000 using the S4 flow cell. The obtained sequencing reads were processed using the Dynamic Read Analysis for GENomics Bio-IT (DRAGEN, Illumina Inc.) platform. Reads were then mapped and aligned to the human reference genome (GRCh37/hg19). The variant calling was done using Haplotype Caller (DRAGEN) and annotation of merged VCFs (Variant Call Format file) by ANNOVAR (annovar.openbioinformatics.org). In the initial stage of variant prioritization for clinical correlation, variants fulfilling the following criteria were used: rare and deleterious with minor allele frequency (MAF) of < 0.01% in the population databases of 1000 Genomes Project (1KGP), Exome Aggregation Consortium (ExAC), and Genome Aggregation Database (gnomAD). Here, the estimated extent of deleteriousness of each variant was calculated using the cumulative sum of prediction scores assigned by computational tools, i.e., PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (https://sift.bii.a-star.edu.sg/) and Mutation taster v2 (http://www.mutationtaster.org/ChrPos.html). Next, variants in the 210 candidate genes (Additional file 1: Table S1) reported for cardiomyopathy phenotype were further prioritized for screening. Variants finally selected for each patient were then classified using American College of Medical Genetics (ACMG) guidelines for the interpretation of sequence variants obtained from the Varsome database (https://varsome.com) [10, 11]. Additional prediction scores extracted from Varsome for the finalized variants were BayesDel addAF score, BayesDel addAF prediction, DANN score, EIGEN and EIGEN PC scores, FATHMM-MKL and MutationTaster, as well as conservation scores such as PhastCons100Way and PhyloP100way. Variant-wise values are mentioned in detail in Additional file 1: Table S2 in the Additional file.

Statistical analyses

Descriptive statistics for continuous variables are presented as medians and ranges; categorical variables are presented as frequencies and percentages.

Result

Over the 2-year study period, 71 cases of suspected cardiomyopathy were screened for enrollment. After excluding those with secondary causes, 20 patients were enrolled in the study. Figure 1 shows the flow chart of enrollment of patients in the study. The most common cause of exclusion was lack of consent or death of patients before being enrolled in the study. Another large group of patients excluded had suspected myocarditis. These children presented with LV dysfunction, but LV dilatation did not meet the inclusion criteria for DCM and/or definite history of associated acute viral illness was present suggestive of myocarditis; 4 infants with HCM were excluded as they were diagnosed with Pompe's disease. One patient with hypoglycemia and metabolic acidosis was excluded from the study as she was diagnosed with fatty acid oxidation defect based on an acylcarnitine profile by TMS; 2 patients who fulfilled the criteria for LV non-compaction, as well as dilated cardiomyopathy, were included in the DCM group. There was one patient diagnosed with RCM and another with HCM. No cases of arrhythmogenic right ventricular dysplasia were identified. DCM was the most common morphologic diagnosis within this cohort; 9 patients were less than 2 years of age at enrollment. After a median of 15 months of follow-up, 8 patients had died (all with DCM). Three patients of DCM were not available for a follow-up after the enrollment. Mortality was 44.4% for the overall group and 53.3% (8/15) for the DCM group; 3 patients had sudden cardiac death, and the remaining 5 died from progressive cardiac failure. Ejection fraction had improved by more than 10% in 26.7% (4/15) of the DCM patients. The median time from symptom onset to death was 16.5 months (5–42 months). Table 1 shows the clinical features, selected echocardiography data, and outcomes of the patients included in the study.

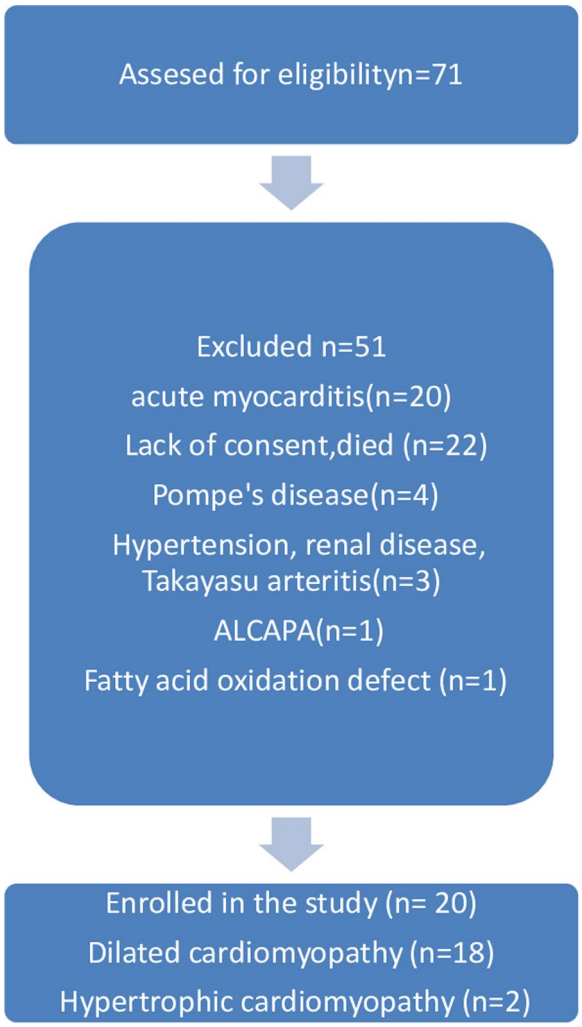

Fig. 1 Flow chart showing enrollment of patients

First-degree relatives were screened based on history, physical examination, and echocardiography. Figure 2 shows flow chart showing family screening of first-degree relatives of index cases. Three-generation family history information was taken into account for all enrolled patients. Screening was indicated in 62 first-degree relatives, of which 40 were parents (median age 29.5 years) and 22 siblings (median age 7 years). Only 38 (61.3%) underwent complete screening including echocardiography and included 31 parents and 7 siblings.

Only 1 patient was identified to have cardiomyopathy on family screening. This patient was an identical twin of a patient with DCM. He was asymptomatic at the time of screening.

Of the 20 patients enrolled for the screening and genetic testing, 18 (16 DCM, 1 RCM, and 1 HCM) underwent multi-panel gene testing. None had a pathogenic or likely pathogenic variant identified by this method.

Among the 9 patients who underwent WES, 5 patients had either pathogenic or likely pathogenic variants as per ACMG-AMP classification (Table 2 and Additional file 1: Table S2). In these 9 patients, overall, there were a total of 8 variations found in 6 different genes: MYBPC3, MYLK3, GATA6, DES, TTN, and DSP.

The flowchart for variant filtering after whole-exome sequencing is represented in Additional file 1: Fig. S1. Out of the nine identified variants, eight different types of variants were identified as follows: three variants were found to be missense (37.5%), four variants were found to be frameshift deletions leading to protein truncation (50% with one repeated), and one variant was found to be nonsense (12.5%).

Table 1 Presenting clinical, echocardiography features, and outcome data

Variables	All patients *n* = 20	DCM *n* = 18
Age at enrollment in months median (range)	42 (6–204)	29.5 (6–132)
Male *n* (%)	10 (50)	10 (55.5)
Ross score at enrollment n (%)		
I	4 (20)	4 (22.2)
II	12 (60)	11 (61.1)
III/IV	4 (20)	3 (16.7)
History of edema *n* (%)	9 (45)	7 (38.9)
History of Syncope *n* (%)	3 (15)	2 (11.1)
Median LVEF % (range)	34 (15–68)	32 (15–44)
Median LVIDd z score (range)	3.75 (− 1.86 to 8.6)	3.95 (2.5–8.6)
Features of diastolic dysfunction *n* (%)	4 (20)	2 (11.1)
Median duration of follow-up in months (range)	15 (0.5–20)	15 (0.5–20)
Mortality at the end of the study	44.4% (8/18)	53.3% (8/15)

DCM Dilated cardiomyopathy, *LVEF* Left ventricular ejection fraction, *LVIDd* Left ventricular internal dimension in diastole

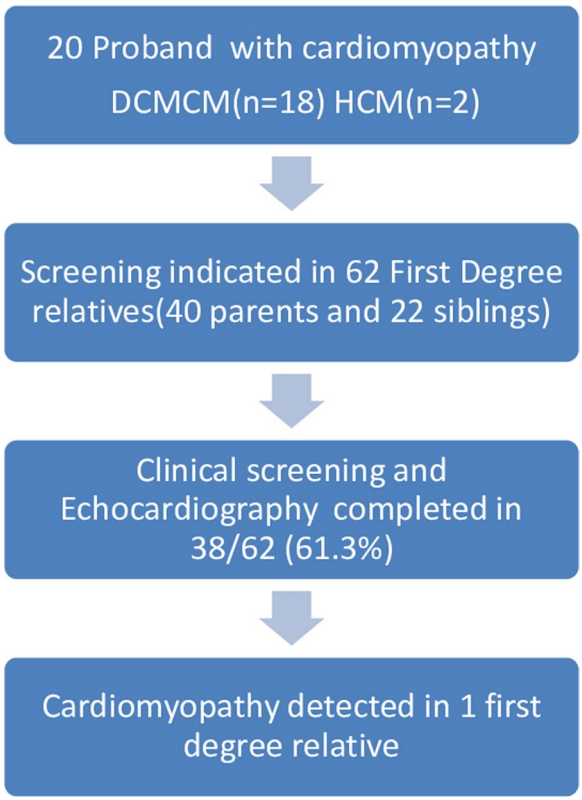

Fig. 2 Flow chart showing family screening of first-degree relatives of index cases

MYBPC3 was the most commonly involved gene. A frameshift deletion in the MYBPC3 gene (i.e., MYBPC3(NM_000256.3):c.2610del(p.Ser871AlafsTer8) was observed in 2 different patients. Although the variant identified in the MYLK3 gene has been classified as 'Likely Pathogenic' according to ACMG classification, no previous publications support its pathogenicity. All but one of these variations have been previously reported (Additional file 1: Table S2). The novel variant identified in GATA6 has been classified as 'Likely Pathogenic' according to ACMG classification.

Discussion

In this study, we investigated the morphological spectrum, genetic/familial causes, and short-term outcome of idiopathic primary cardiomyopathy in 20 unrelated children presented to a tertiary care hospital in North India. DCM was the most common morphologic diagnosis (90% of patients), and these patients had an adverse short-term outcome. Previously, population-based studies with larger cohorts have also shown that DCM is the most common morphology of cardiomyopathy [1–3].

Among the 17 patients who were followed up (there were three losses to follow-up), the mortality rate after a median of 15 months was 53.3% for the DCM group. Previous studies have shown that nearly 40% of children who exhibit symptomatic cardiomyopathy undergo heart transplantation or die within the first 2 years after diagnosis [12]. The most common mode of death was worsening heart failure. Improvement in ejection fraction occurred in 26.7% of DCM patients over time and was consistent with normalization rates in a previous PCMR (Pediatric Cardiomyopathy Registry) study [12].

Current guideline recommends three-generation family history evaluation, clinical screening of relatives, and counselling along with genetic testing in patients with idiopathic cardiomyopathy [4].

Clinical phenotype screening with echocardiography was done in 61.3% of the eligible first-degree relatives in our study. Only 5% (1/20) had familial cardiomyopathy. Previous studies in children, reported from Western countries, have found a slightly higher prevalence of familial disease [2, 13–15]. The prevalence of familial cardiomyopathy varies across studies depending upon the definition used, age group of patients, morphology of cardiomyopathy, prospective/retrospective design, and methods of investigations, especially the type of imaging used for screening.

There could be a variety of reasons for the low prevalence of familial cardiomyopathy in our study. Firstly, clinical phenotype screening was done only in 61.1% of eligible first-degree relatives. Secondly, we did not have access to medical records of the relatives of patients, and hence, familial etiology may have been underreported. Lastly, we screened relatives at only one point during the study. Screening of family members needs to be done periodically as family members who are asymptomatic at the time of testing may develop cardiomyopathy at a later stage.

In total, 5 out of 9 patients who underwent whole-exome sequencing in our study had either pathogenic or likely pathogenic variants, with one variant being shared by 2 patients. Variants identified in our study are largely in the MYBPC3 gene. MYBPC3 accounts for up to 10% of cases of DCM [16]. However, in our study, MYBPC3 variants contributed to 4 out of 9 cases (44.4%), of which two patients share the same variant (MYBPC3(NM_000256.3):c.2610del(p.Ser871AlafsTer8). Our small cohort size may explain this large deviation in frequency of MYBPC3 variants from previous reports and is not a result of the difference in ethnicity of patients as compared to other studies. The expectation that variant frequency in the MYBPC3 gene should not digress drastically in the Indian population is supported by the similar pLoF (predicted Loss-of-Function) allele frequencies for MYBPC3 across various ethnic groups. (https://varsome.com/gene/hg19/MYBPC3).

Table 2 Genotype and phenotype characteristics in those who underwent WES

SN	Age (months)/ gender	Diagnosis	Current Status	Result of whole-exome sequencing				AGGRESCAN	PhosphoSite	SpliceAILookup	Conservation score
				Coordinate	Amino acid change	Type of variant	ACMG variant classification				PhyloP100way
Pathogenic and likely pathogenic variants (P/LP)											
1	48/F	DCM	Deceased	chr11:47,364,158 C > -	MYBPC3(NM_000256.3):c.1595del(p.Gly532AlafsTer23)	Frameshift deletion	P	0	No	No	7.234, 5.542, −3.351, 7.234, 7.234
2	12/F	DCM	Alive	chr11:47,357,555 G > -	MYBPC3(NM_000256.3):c.2610del(p.Ser871AlafsTer8)	Frameshift deletion	P	0	No	No	−0.511, 9.426, 3.950, 1.163, 8.735, 5.972
3	9/M	DCM	Deceased	chr11:47,357,555 G > -	MYBPC3(NM_000256.3):c.2610del(p.Ser871AlafsTer8)	Frameshift deletion	P	0	No	No	−0.511, 9.426, 3.950, 1.163, 8.735, 5.973
4	8/M	DCM	Deceased	chr16:46,766,372 G > A	MYLK3(NM_182493.3):c.1210C > T (p.Gln404Ter)	Nonsense mutation	LP	−8.7	No	No	0.832
5	9/M	DCM	Lost to follow-up	chr18:19,751,370 G > -	GATA6(NM_005257.6):c.268del(p.Ala90LeufsTer47)	Frameshift deletion	LP	0	No	No	5.388, 2.644, 0.797, 1.053
Variants of unknown significance (VUS)											
6	23/M	DCM with non-compaction	Alive	chr11:47,365,047 C > T	MYBPC3(NM_000256.3):c.1219G > A(p.Gly407Ser)	Missense mutation	VUS	0	Phosphorylation on S406 and S408	Donor loss—0.04—0 bp, Donor Gain—0.04—-4 bp	5.454
7	54/F	DCM with non-compaction	Alive	chr2:179,496,966 G > A	TTN(NM_001267550.2):c.43655C > T(p.Ser14552Leu)	Missense mutation	VUS	NA	Ubiquitination on R14553	Donor Gain—0.13—36 bp	5.57
8	13/M	DCM	Lost to follow-up	chr2:220,283,700 GC > -	DES(NM_001927.4):c.525_526del(p.Val176ArgfsTer48)	Frameshift deletion	VUS	0	No	Donor Gain—0.04—-18 bp	4.168, 3.658, 6.527, −0.140, 2.346, 5.820, −0.336, 2.916, 9.537, −0.508, 7.699
9	15/M	DCM	Deceased	chr6:7,566,613 C > T	DSP(NM_004415.4):c.943C > T(p.Arg315Cys)	Missense mutation	VUS	0.1	No	No	6.999

The identified variants have been listed along with the HGVS nomenclature, ACMG classification, and corresponding valid rule and supporting information from other computational prediction tools, i.e., AGGRESCAN, PhospoSite, and SpliceAILookup. Detailed scores of all computational tools obtained from Varsome are included in Table S2 in the Additional file 1 to support its pathogenicity. AGGRESCAN is a web-based software used for the prediction of aggregation-prone regions in protein sequences, the analysis of the effect of variants on protein aggregation tendencies, and for the comparison of the aggregation properties of different proteins. Negative values suggest low aggregation propensity, and vice versa for positive values. PhosphoSite is a curated, web-based bioinformatics resource devoted to protein phosphorylation sites in human and mouse genomes. It provides information about the phosphorylated amino acid and its surrounding sequence, location of the site within known domains and motifs, orthologous sites in other species, as well as relevant literature references. SpliceAILookup is an open-source deep learning-based splicing prediction tool used to identify splice variants and annotates genetic variants with their predicted effect on splicing. PhyloP100way scores are based on multiple alignments of 99 vertebrate genome sequences to the human genome. The higher the score, the more conserved the site

DCM Dilated cardiomyopathy, *MYBPC* myosin-binding protein C, *MYLK* myosin light chain kinase, *GATA6* GATA-binding protein 6, *TTN* titin, *DES* desmin, *DSP* desmoplakin, *P* pathogenic, *LP* likely pathogenic, *VUS* variant of unknown significance

Importantly, the identification of a pathological variant does not necessarily have similar prognostic implications as exemplified by two patients in our study who shared the same variant on the MYBPC3 gene, (MYBPC3(NM_000256.3):c.2610del(p.Ser871AlafsTer8). One of these patients died at 9 years old, while the second patient is still alive at 12 years of age. Accurate interpretation and determination of the clinical significance of the variants are vital, given the serious consequences on the patient and their loved ones.

Variants in TTN which are reported to be the commonest genetic cause of DCM contribute to up to 14% of cases [17, 18]. This was seen in only one patient in our study. TTN (TTN(NM_001267550.2):c.43655C > T(p.Ser14552Leu)) was a novel variant found in this patient and was classified as a VUS according to several prediction scores.

Genetic heterogeneity is highly pronounced in cardiomyopathies, with more than 40 genes being implicated. This is complicated further by the fact that a majority of these genes only account for a small percentage of cases [17]. Moreover, there are issues like incomplete or age-related penetrance and variable expressivity also add to diagnostic difficulties. None of our patients had any pathogenic variants detected with the use of a multi-gene panel which included 17 genes. Newer genes and variants causing dilated cardiomyopathy are detected with increasing frequency, and therefore, any genetic panel is likely to be outdated quickly. We observed considerable heterogeneity in variants from patient to patient and found that WES was a useful diagnostic technique for identifying genetic causes of DCM. However, our study was not designed to compare WES and genetic panel testing, and therefore, no conclusion can be derived from our results regarding the superiority of one method over another.

There is contradictory evidence in the literature regarding the use of WES as a primary method in the diagnosis of genetic cardiomyopathy. Some studies have supported the use of WES [19–21]. However, there is a fear that increasing use of exome sequencing may result in a greater proportion of children being detected to have variants of unknown significance or increase the group of patients with "genotype-positive/phenotype-negative". One recent study did not support the use of WES as a primary tool in dilated cardiomyopathy [22]. However, they did not compare their results with multi-panel gene testing.

Future studies should systematically study the efficacy and cost-effectiveness of WES as a primary diagnostic tool and compare it with multi-panel genetic testing.

Conclusion

Dilated cardiomyopathy is the commonest morphologic subtype of pediatric cardiomyopathy in our region and has high short-term mortality. The familial cause was found in 5% by clinical screening of first-degree relatives and was less than reported previously. Emphasis on clinical phenotype screening of first-degree relatives should be continued and pursued more thoroughly; 5 out of 9 (55%) who underwent testing had a pathogenic or likely pathogenic variant identified in whole-exome sequencing. Future studies should evaluate the role of whole-exome sequencing in identifying genetic causes of cardiomyopathy in children.

Strength of the study

Familial cardiomyopathy was diagnosed based on clinical as well as echo screening of first-degree relatives and was not solely based on a history of cardiomyopathy in other family members. Whole-exome testing was done (in a subset of patients) to identify a genetic cause of cardiomyopathy.

Limitations of the study

This was a small study. The exclusion of viral myocarditis was based on history. Myocardial biopsy or MRI was not done on any patient to rule in or rule out myocarditis. Investigations to rule out metabolic causes were based on clinical suspicion and were not done for all patients. Whole-exome sequencing was not done for all patients, and patients selected to undergo exome sequencing were arbitrary. Genotype–phenotype correlation was not done as our sample size was too small for any meaningful evaluation. However, we have listed the phenotypic features along with their genotype in Additional file 1: Table S3. Another limitation of the present study is the absence of a control set/population, making it difficult to differentiate true causative variants from incidental findings by case–control statistical evaluation of genotype–phenotype association.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43042-023-00414-0.

Additional file 1. Candidate genes reported for cardiomyopathy phenotype and details of pathologic variant information identified in the study along with their clinical phenotype.

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None.

Author contributions
DB, DK, and FM involved in conception and design of the study. DB, SM, VA, US, BU, DK, and FM involved in acquisition, analysis, and interpretation of data. SB involved in the analysis, interpretation of data, and in the revision of the original manuscript. DB, DK, and FM drafted the work and its revision. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The authors assert that all procedures contributing to this work comply with the ethical standards of the Indian council of medical research and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the Institutional Ethics Committee of PGIMER and DR RML hospital, New Delhi India. (No. 196(15/2017)/IEC/PGIMER/RMLH)454/18) and IGIB (No. IGIB/IHEC/GOMED/2022-2023).

Consent for publication
Written informed consent to publish was obtained from the parents of the children who participated in the study.

Competing interests
The authors declare that they have no competing interests.

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