Cardiology in the Young

cambridge.org/cty

Molecular analysis of dilated and left ventricular noncompaction cardiomyopathies in Egyptian children

Dina A. Mehaney[1,2,*], Alireza Haghighi[3,4,5,6,*], Amira K. Embaby[1], Reham A. Zeyada[1], Rania K. Darwish[1,2,*], Nesrine S. Elfeel[7], Mohamed Abouelhoda[8,*], Sonia A. El-Saiedi[7,**], Nadida A. Gohar[1,**] and Zeinab S. Seliem[7,**]

[1]Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt; [2]Next Generation Sequencing Laboratory, Cairo University Children Hospital, Egypt; [3]Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [4]Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [5]Department of Genetics, Harvard Medical School, Boston, MA, USA; [6]Howard Hughes Medical Institute, Brigham and Women's Hospital, Boston, MA, USA; [7]Pediatrics Department, Faculty of Medicine, Cairo University, Cairo, Egypt and [8]Systems and Biomedical Engineering Department, Faculty of Engineering, Cairo University, Cairo, Egypt

Original Article

Cite this article: Mehaney DA, Haghighi A, Embaby AK, Zeyada RA, Darwish RK, Elfeel NS, Abouelhoda M, El-Saiedi SA, Gohar NA, and Seliem ZS (2022) Molecular analysis of dilated and left ventricular noncompaction cardiomyopathies in Egyptian children. *Cardiology in the Young* **32**: 295–300. doi: 10.1017/S1047951121002055

Received: 29 January 2021
Revised: 8 April 2021
Accepted: 30 April 2021
First published online: 26 May 2021

Keywords:
Dilated cardiomyopathy; left ventricular noncompaction cardiomyopathy; next-generation sequencing; paediatrics; Egypt

Author for correspondence: Dr D. A. Mehaney, MD, Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Kasr Alainy St., Cairo, 11562, Egypt. Tel: +201023123423; Fax: +2023644383. E-mail: drdinamehaney@kasralainy.edu.eg

*Dina A. Mehaney, Alireza Haghighi, Rania K. Darwish, and Mohamed Abouelhoda are first authors on this review with a shared first co-authorship.

**Sonia A. El-Saiedi, Nadida A. Gohar, and Zeinab S. Seliem Elhoda are the last authors on this review with a shared last co-authorship.

Abstract

Background: Paediatric cardiomyopathy is a progressive, often lethal disorder and the most common cause of heart failure in children. Despite its severe outcomes, the genetic aetiology is still poorly characterised. High-throughput sequencing offers a great opportunity for a better understanding of the genetic causes of cardiomyopathy. *Aim:* The current study aimed to elucidate the genetic background of cardiomyopathy in Egyptian children. *Methods:* This hospital-based study involved 68 patients; 58 idiopathic primary dilated cardiomyopathy and 10 left ventricular noncompaction cardiomyopathy. Cardiomyopathy-associated genes were investigated using targeted next-generation sequencing. *Results:* Consanguinity was positive in 53 and 70% of dilated cardiomyopathy and left ventricular noncompaction cardiomyopathy patients, respectively. Positive family history of cardiomyopathy was present in 28% of dilated cardiomyopathy and 10% of the left ventricular noncompaction cardiomyopathy patients. In 25 patients, 29 rare variants were detected; 2 likely pathogenic variants in *TNNI3* and *TTN* and 27 variants of uncertain significance explaining 2.9% of patients. *Conclusions:* The low genetic detection rate suggests that novel genes or variants might underlie paediatric cardiomyopathy in Egypt, especially with the high burden of consanguinity. Being the first national and regional report, our study could be a reference for future genetic testing in Egyptian cardiomyopathy children. Genome-wide tests (whole exome/genome sequencing) might be more suitable than the targeted sequencing to investigate the primary cardiomyopathy patients. Molecular characterisation of cardiomyopathies in different ethnicities will allow for global comparative studies that could result in understanding the pathophysiology and heterogeneity of cardiomyopathies.

Paediatric cardiomyopathy has an incidence of 1.1–1.5:100,000.[1] Although rare, paediatric cardiomyopathy carries a major risk of morbidity and mortality.[2] Data from international paediatric dilated cardiomyopathy registries indicate that the rates of heart transplantation or death over 1- and 5-year periods are 31 and 46%, respectively.[3] Elmasry et al reported the annual incidence of paediatric cardiomyopathy to be 1.24 per 100,000 Egyptian children <10 years.[4] In Egyptian hospital-based paediatric studies, dilated cardiomyopathy was the most common phenotype of cardiomyopathy, representing 76[5] to 88.7%[4] of patients.

Dilated cardiomyopathy (OMIM 115200) is classified as idiopathic when all detectable causes had been excluded, it accounts approximately for 50% of cases.[6] Pathogenic variants in many genes involved in cardiac functions accounted for a significant proportion of idiopathic dilated cardiomyopathy.[7] It is estimated that approximately 30–50% of dilated cardiomyopathy patients have a known genetic cause.[8] Dilated cardiomyopathy is heterogeneous, and mutations have been reported in >50 genes.[9] However, most genetic studies have been focused on dilated cardiomyopathy in adult.[10]

Left ventricular noncompaction cardiomyopathy (OMIM 604169) is the third most common cardiomyopathy phenotype.[11] The prevalence of left ventricular noncompaction in the paediatric age group is around 0.14%.[12] Studies of adult and paediatric left ventricular noncompaction cohorts reported that 18–44% of cases[13] have a disease-causing variant; however, the underlying cellular and molecular mechanisms are not fully understood.

CAMBRIDGE UNIVERSITY PRESS

Genetic disorders are a great public health problem in Egypt. The consanguinity is prevalent in Egypt, up to 86% among first cousins.[14] In an Egyptian study of 50 children with paediatric cardiomyopathy, 64% of patients were consanguineous, and a history of similar disease in the family was present in 22% of patients.[5]

There is no report about the genetic basis of dilated cardiomyopathy and left ventricular noncompaction in Egyptian children. Dilated cardiomyopathy was the most common cardiomyopathy phenotype (72%) at the Pediatric Cardiomyopathy Clinic of Cairo University Children's Hospital, whereas left ventricular noncompaction accounted for 2.7% of patients.[15] Due to limited genetic testing at Cairo University Children's Hospital, the patients were not genetically tested.

Next-generation sequencing has become an important part of the clinical workup of cardiomyopathies and is now included in clinical guidelines.[16] By establishing next-generation sequencing technology in Cairo University Children's Hospital, the current study aimed at elucidating the genetics of dilated cardiomyopathy and left ventricular noncompaction in a cohort of Egyptian children.

Materials and methods

The current pilot study included 68 unrelated patients aged <16 years who presented to the Cairo University Children's Hospital with primary idiopathic dilated cardiomyopathy (58 patients) or left ventricular noncompaction (10 patients). Patients with two subtypes of left ventricular noncompaction were included: (a) isolated left ventricular noncompaction and (b) co-occurring with dilated cardiomyopathy or hypertrophic cardiomyopathy. Patients older than 16 years and those with myocarditis, severe concomitant disease, hypertension, ischaemia, primary valvular heart disease, or a history of cardiotoxic drug exposure were excluded from the study. Patients with underlying cardiac hypertrophy or with extracardiac manifestations were also excluded. Methods for clinical genetic workup and bioinformatic data analysis have been described previously.[17]

Clinical workup included (a) documenting the family history and pedigree. If two or more closely related family members were affected, they were classified as familial[18]; (b) documenting the medical history with the assessment of heart failure symptoms using ROSS/NYHA classification[19]; (c) full physical and cardiac examination; and (d) radiological investigations including chest X-ray and two-dimensional echocardiography. Patients were diagnosed as idiopathic dilated cardiomyopathy according to the definition by World Health Organization/International Society and Federation of Cardiology,[20] and the diagnosis of left ventricular noncompaction was made using previously described criteria[21]; (e) 12-lead electrocardiogram to rule out arrhythmias; and (f) other tests such as metabolic screen and enzymatic assays for the exclusion of inborn errors of metabolism.

Next-generation sequencing and bioinformatic analysis

The Illumina MiSeq system and the TruSight Cardio panel (Illumina, San Diego, CA, United States of America) were used for targeted next-generation sequencing. Following data demultiplexing and generating FASTQ files by the MiSeq Reporter software, the Burrows-Wheeler Aligner was used to align the reads against the hg19 human reference genome. The Genome Analysis Toolkit[22] was used for variant calling. The variant call files were annotated using ANNOVAR,[23] Human Gene Mutation Database *(HGMD Professional 2019.4)*, Online Mendelian Inheritance in Man (OMIM), and ClinVar. The variants were annotated with *in silico* predictions including SIFT, PolyPhen-2, and Combined Annotation Dependent Depletion (CADD). The variants were filtered for minor allele frequency in general population using 1000 genome database, dbSNP (V138), the Genome Aggregation Database (gnomAD v2.1), and the Middle Eastern databases.[24,25] Variants with minor allele frequencies >0.05% were excluded. Variants were considered very rare and rare if they have minor allele frequencies <0.01 and <0.05%, respectively. The pathogenicity of variants was assessed according to the guidelines of the American College of Medical Genetics and Genomics.[26] Sanger sequencing was used to confirm the identified variants.

Results

In total, 58 dilated cardiomyopathy patients (32 females and 26 males) and 10 left ventricular noncompaction patients (8 males and 2 females) were investigated. Six patients had isolated left ventricular noncompaction, two had left ventricular noncompaction and dilated cardiomyopathy, and two had left ventricular noncompaction and hypertrophic cardiomyopathy. The median (range) age of the dilated cardiomyopathy and left ventricular noncompaction patients was 3.5 years (2.4 months – 16 years) and 5.25 years (6 months – 14 years), respectively. The age of initial presentation was <2 years in 51% of dilated cardiomyopathy and 80% of left ventricular noncompaction patients. Consanguinity was present in 53% of dilated cardiomyopathy and 70% of left ventricular noncompaction patients. Totally, 28% of dilated cardiomyopathy patients had a positive family history of cardiomyopathy, compared to 10% of the left ventricular noncompaction patients. The most common clinical presentation was heart failure. Dyspnea was the most common symptom followed by recurrent respiratory infections. The demographic, clinical, and cardiac evaluation data of the dilated cardiomyopathy and left ventricular noncompaction patients are summarised in Supplementary Table S1.

Nineteen variants were identified in 18 dilated cardiomyopathy patients. Nine novel variants were not previously reported in the literature, ClinVar, or gnomAD (Table 1). Two likely pathogenic variants were detected in dilated cardiomyopathy patients, *TNNI3*: c.610C>T (p.Arg204Cys) and *TTN*:c.104269C>T (p.Gln34757*). The other 17 variants were classified as variants of uncertain significance per the American College of Medical Genetics guidelines.[26] Those variants of uncertain significance were rare and found to be deleterious by *in silico* prediction algorithms. One patient (#43) had a homozygous variant, *JPH2*:c.1426G>T (p.Glu476*), and one patient (#7) had compound heterozygous variants in *MYH7* and *VCL*. Table 1 includes all the rare (minor allele frequency < 0.05%) variants. Here, we describe the very rare (minor allele frequency <0.01%) variants that we identified.

A likely pathogenic variant in *TNNI3*, c.610C>T (p.Arg204Cys) (ClinVar ID: 177631), was identified in a male dilated cardiomyopathy patient (#85) presented at the age of 2 years with dyspnea and heart failure. The patient was born to consanguineous parents with normal parental echocardiography. His brother and sister had dilated cardiomyopathy and restrictive cardiomyopathy, respectively. Therefore, the condition was classified as familial dilated cardiomyopathy. This variant was reported previously in hypertrophic cardiomyopathy patients[27–29] but was absent from gnomAD. This variant affects a highly conserved amino acid. Functional studies confirmed its deleterious effect on the interactions of cardiac troponin.[30] Another missense variant in the same codon *TNNI3*: Arg204His has been reported in association with hypertrophic

https://doi.org/10.1017/S1047951121002055 Published online by Cambridge University Press

Table 1. List of identified variants in DCM and LVNC patients

Patient No. (Phenotype)	GT	Variant	rs ID	gnomAD Pop-Max	SIFT	PolyPhen 2	CADD	Classification	ClinVar ID
85 (DCM)	Het	*TNNI3* (NM_000363.4, exon 8): c.610C>T (p.Arg204Cys)	rs727504243	.	0 (D)	1 (D)	34	LP	177631
13 (DCM)	Het	*TTN* (NM_001267550, exon 358): c.104269C>T (p.Gln34757*)	.	.	.	.	69	LP	.
28 (DCM)	Het	*TTN* (NM_001267550, exon 30): c.7006T>C (p.Tyr2336His)	.	.	0.009 (D)	1 (D)	22.1	VUS	.
31 (DCM)	Het	*TTN* (NM_001267550, exon 326): c.74927G>C (p.Gly24976Ala)	.	.	0.01 (D)	1 (D)	20.1	VUS	.
142 (DCM)	Het	DSG2 (NM_001943.5, exon 8): c.880A>G (p.Lys294Glu)	rs752432726	0.00026	0.009 (D)	0.97 (D)	23.3	VUS	199803
166 (DCM)	Het	*TTN* (NM_001267550, exon 288): c.56018C>A (p.Thr18673Asn)	.	.	0.04 (D)	0.99 (D)	20.5	VUS	.
190 (DCM)	Het	*TTN* (NM_001267550, exon 344): c.95459G>A (.p.Gly31820Asp)	rs1340172871	.	0.02 (D)	0.98 (D)	22.8	VUS	.
109 (DCM)	Het	*MYH7* (NM_000257, exon 23): c.2821C>T (p.Arg941Cys)	.	.	0.018 (D)	1 (D)	34	VUS	925464
112 (DCM)	Het	*MYH7* (NM_000257, exon 30): c.4076G>A (p.Arg1359His)	rs750836033	0.00013	0 (D)	0.995 (D)	20.4	VUS	228910
139 (DCM)	Het	*MYH7* (NM_000257, exon 18): c.2008G>C (p.Val670Leu)	.	.	0 (D)	0.986 (D)	29.5	VUS	.
7 (DCM)	Het	*MYH7* (NM_000257, exon 28): c.3749G>A (p.Arg1250Gln)	rs540263945	0.0001	0.001 (D)	0.984 (D)	22.5	VUS	.
	Het	*VCL* (NM_014000, exon 9): c.1040C>A (p.Pro347Gln)	.	.	0.05 (T)	0.895 (P)	33	VUS	.
97 (DCM)	Het	*LAMA4* (NM_001105206, exon 30): c.4075C>T (p.Pro1359Ser)	.	.	0.008 (D)	0.999 (D)	27.8	VUS	.
40 (DCM)	Het	*RBM20* (NM_001134363, exon 9): c.2147G>A (p.Arg716Gln)	rs375798246	0.00035	0.0003 (D)	0 (B)	24.5	VUS	43986
49 (DCM)	Het	*TPM1* (NM_001018008, exon 2): c.214A>G (p.Thr72Ala)	.	.	0.03 (D)	0.989 (D)	20.2	VUS	.
43 (DCM)	Hom	*JPH2* (NM_020433, exon 4): c.1426G>T (p.Glu476*)	rs1259828095	0.00038	.	.	37	VUS	.
67 (DCM)	Het	*TNNT2* (NM_001276345.2, exon 12): c.503G>A (p.Arg168Gln)	rs397516468	.	0.001 (D)	1 (D)	35	VUS	43645
175 (DCM)	Het	*BAG3* (NM_004281, exon 3): c.743A>G (p.His248Arg)	rs369947845	0.00031	0.02 (D)	0.506 (P)	25	VUS	201687
169 (DCM)	Het	*TPM1* (NM_001018005, exon 5): c.533G>A (p.Arg178His)	rs397516375	.	0 (D)	1 (D)	35	VUS	43423
64 (LVNC)	Het	*ACTC1* (NM_005159, exon 6): c.970C>T (p.Pro324Ser)	rs886038880	.	.	0.94 (D)	29.7	VUS	263668
136 (LVNC)	Het	*MYH7* (NM_000257, exon 30): c.4042G>A (p.Glu1348Lys)	rs1275262402	0.0002	0 (D)	0.924 (D)	22.6	VUS	524974
	Het	*MYH7* (NM_000257, exon 24): c.2997delG (p.Leu1000fs)	.	.	.	.	.	VUS	.

(*Continued*)

https://doi.org/10.1017/S1047951121002055 Published online by Cambridge University Press

Table 1. (*Continued*)

Patient No. (Phenotype)	GT	Variant	rs ID	gnomAD Pop-Max	SIFT	PolyPhen 2	CADD	Classification	ClinVar ID
277 (LVNC)	Het	*TTN* (NM_001267550, exon 327): c.87008C>T (p.Ser29003Phe)	rs754378461	0.00007	0.037 (D)	0.927 (D)	19.01	VUS	.
163 (LVNC +DCM)	Het	*TTN* (NM_001267550, exon 226): c.41503C>T (p.Arg13835Trp)	.	.	0.003 (D	0.984 (D)	14.2	VUS	.
	Het	*MYPN* (NM_032578, exon 18): c.3548T>C (p.Val1183Ala)	rs878855166	.	0.018 (D)	0.999 (D)	24	VUS	241710
223 (LVNC +DCM)	Hom	*TNNI3* (NM_000363.5 (exon 5): c.258delC (p.Leu88fs)	rs727503507	0.00007	.	.	.	VUS	165522
205 (LVNC +HCM)	Het	*TTN* (NM_001267550, exon 304): c.62681G>T (p.Cys20894Phe)	rs370080086	0.00021	0.003 (D)	1 (D)	22.7	VUS	.
	Het	*DTNA* (NM_001390, exon 3): c.362C>T (p.Pro121Leu)	rs104894654	0.00016	0.253 (T)	0.898 (p)	22.6	VUS	8306
130 (LVNC +HCM)	Het	*TTN* (NM_001267550, exon 335): c.90238G>A (p.Ala30080Thr)	rs753375022	0.000068	0.036 (D)	1 (D)	23.2	VUS	.

GT: Genotype, Het: heterozygous, Hom: homozygous, LP: Likely pathogenic, VUS: variant of uncertain significance, HCM: hypertrophic cardiomyopathy, DCM: dilated cardiomyopathy, LVNC: left ventricular noncompaction.

cardiomyopathy[27] and restrictive cardiomyopathy[31] and was classified as pathogenic suggesting the critical importance of Arg204 for the function of *TNNI3*. Therefore, the other missense variants at this position are more likely to be pathogenic.

A stop variant was identified in *TTN*, c.104269C>T (p.Gln34757*), in a female dilated cardiomyopathy patient (#13) who presented at birth with recurrent attacks of chest infection and dyspnea. The patient eventually developed heart failure. She was born to consanguineous parents with a normal cardiac examination. This loss of function variant is absent from general population databases and has not been previously reported in the literature. Truncating variants in *TTN* are only known to cause dilated cardiomyopathy when found in exons constitutively expressed in the heart (proportion spliced in>0.9). This variant is located in exon 358 (A band with PSI = 1) of the gene and is predicted to introduce a premature stop codon at position 34,757 of the titin protein. Therefore, we classified this variant as likely pathogenic.

We identified two variants of uncertain significance in *MYH7* (patients #109 and #7). c.2821C>T (p.Arg941Cys) was identified in a male patient (#109) who presented with a recurrent chest infection at the age of 6 months. He was born to non-consanguineous parents with a normal cardiac examination. This variant was previously reported in one Chinese left ventricular noncompaction patient with heart failure[32] and in a Japanese paediatric left ventricular noncompaction patient with heart failure whose father had dilated cardiomyopathy.[33] p.Arg941Cys has been reported in ClinVar as a variant of uncertain significance (ID 925464). Also, it was reported in a compound heterozygote state in a 66-year-old female with hypertrophic cardiomyopathy and end-stage progression to dilated phenotype. Another variant of uncertain significance in the same codon (p.Arg941Ser) was reported in a hypertrophic cardiomyopathy patient (ClinVar ID: 407201). It has a very low minor allele frequency in population databases.

In a male dilated cardiomyopathy patient (#7), we identified two variants of uncertain significance: *MYH7* c.3749G>A (p.Arg1250Gln) in *MYH7* and c.1040C>A (p.Pro347Gln) in *VCL*. This patient presented with a recurrent chest infection, dyspnea, and heart failure and was diagnosed with dilated cardiomyopathy at the age of 3 years. His father had dilated cardiomyopathy and left ventricular noncompaction. c.3749G>A occurs at a conserved nucleotide (p.Arg1250Gln), and *in silico* algorithms predicted that it is deleterious. A different missense variant (p.Arg1250Trp) in the same residue was reported in an African American patient with left ventricular noncompaction and dilated cardiomyopathy and two family members with dilated cardiomyopathy.[34] c.1040C>A (p.Pro347Gln) in *VCL* was not previously reported in the literature; however, another rare variant in the same codon (p.Pro347Leu) was reported as variant of uncertain significance by clinical testing laboratories in dilated cardiomyopathy and familial hypertrophic cardiomyopathy patients (ClinVar ID: 300786).

The variant *TNNT2*:c.503G>A (p.Arg168Gln) was reported in a female dilated cardiomyopathy patient (#67) who presented since birth with dyspnea and heart failure. She was born to non-consanguineous parents with a normal echocardiogram. This variant was reported by a clinical laboratory and classified as variant of uncertain significance (Clinvar ID: 43645).

A *TPM1* variant, c.533G>A (p.Arg178His), was identified in a 4-year-old male patient (#169), who had presented at the age of 1 year with dyspnea and heart failure and was diagnosed with dilated cardiomyopathy. The patient was born to healthy consanguineous parents. This variant was reported in an infant with heart failure and left ventricular noncompaction[35] and was classified as variant of uncertain significance (ClinVar ID: 43423).

Ten rare variants of uncertain significance were identified in seven left ventricular noncompaction patients (Table 1). They were predicted to be deleterious by *in silico* algorithms. Two variants were novel: *MYH7*:c.2997delG (p.Leu1000fs) *and TTN*: c.41503C>T (p.Arg13835Trp). Compound heterozygous variants were detected in *MYH* (patient #136), *TTN*, *MYPN* (patient#163), *TTN*, and *DTNA* (patient#205) (Table 1).

https://doi.org/10.1017/S1047951121002055 Published online by Cambridge University Press

One homozygous variant in *TNNI3*, c.258del (p.Leu88fs), was reported in a 7-year-old male (patient #223) who presented with dyspnea and heart failure and was diagnosed at the age of 6 months with left ventricular noncompaction and dilated cardiomyopathy. He was born to healthy consanguineous parents. This variant was previously reported in an adult hypertrophic cardiomyopathy patient.[36] It remains unclear if this variant would impact protein function, and therefore it was classified as variant of uncertain significance (ClinVar ID:165522).

ACTC1:c.970C>T (p.Pro324Ser) was identified in a 2.5-year-old male patient (#64) with left ventricular noncompaction who presented at the age of 6 months with dyspnea and heart failure. The patient was born to non-consanguineous healthy parents. p.Pro324Ser was reported by a clinical laboratory in association with a cardiovascular phenotype and classified as variant of uncertain significance (ClinVar ID: 263668).

We identified *MYPN*:c.3548T>C (p.Val1183Ala) in a 14-year-old male patient (#163) who was diagnosed with left ventricular noncompaction and dilated cardiomyopathy at the age of 1 year. The patient was born to healthy consanguineous parents. This variant is absent in gnomAD and has not been reported in the literature. However, it was reported in ClinVar in association with dilated cardiomyopathy (ClinVar ID: 241710). The same patient harboured a *TTN* variant of uncertain significance, c.41503C>T (p.Arg13835Trp) that was not previously reported.

Discussion

In the current study, we analysed cardiomyopathy-associated genes in 68 unrelated paediatric dilated cardiomyopathy and left ventricular noncompaction patients using next-generation sequencing. Among these 68 patients, 29 rare variants were detected in 25 patients. Two variants in *TNNI3* and *TTN* that were likely pathogenic could explain only 2/68 (2.9%) of patients. Twenty-seven variants were of uncertain significance. This detection rate is much lower than 25–50% that was previously reported in other paediatric cohorts from other parts of the world.[10,37] The differences in the detection rates among different studies could be attributed to the different genetic causes, the phenotypic composition of the cohorts, the inclusion of secondary cardiomyopathy, different next-generation sequencing strategies used, and different degrees of stringency for the variant classification criteria. In the majority of children, the genetic role remains unclear.[7] A lower detection rate in the Egyptian patients might be due to different genetic aetiology in this population and possibly the higher rates of consanguinity.

In this study, most of the variants were in *TTN* followed by *MYH7* and *MYPBC3*. *TTN* variants have been implicated in diverse types of cardiomyopathies.[38] Studying *TTN* gene variants was not previously straight-forward due to the size and complexity of the gene; however, the introduction of next-generation sequencing has made it feasible.[39] Truncating variants in the *TTN* gene are estimated to account for ˜25 and 15% of familial and sporadic dilated cardiomyopathy, respectively,[40] whereas the significance of non-truncating variants in *TTN* is not clear.[41]

We identified many rare damaging variants in sarcomere and non-sarcomere genes in the left ventricular noncompaction patients. However, all these variants were classified as variant of uncertain significance. Although genetic analysis has not been a first-line clinical diagnostic method for left ventricular noncompaction, sequencing patients will help us to better understand the disease process and to identify at-risk family members.[11]

Variants of uncertain significance represent a major clinical challenge, as proper genetic diagnosis and genetic counseling may not be provided.[42] In this study, nine dilated cardiomyopathy and two left ventricular noncompaction patients harboured 11 novel variants that were not previously reported. We classified these as variants of uncertain significance according to the American College of Medical Genetics guidelines. Seven out of the 11 patients (63.6%) were born to consanguineous parents. Three of these variants of uncertain significance (27.2%) were reported in patients with positive family history. In addition, 11/37 (29.7%) cases who did not have any variant in the cardiomyopathy gene had a family history of a similar cardiac condition. Besides, the echocardiographic examination resulted in the diagnosis of dilated cardiomyopathy in 17/58 (29.3%) parents, who were not previously aware of such diagnosis. These findings suggest that there is an underestimation of the population frequency of dilated cardiomyopathy/left ventricular noncompaction, as well as the familial pattern among the Egyptian paediatric cardiomyopathy patients. It is important to note that some variants of uncertain significance might be pathogenic; however, we may not have enough evidence (such as segregation analysis and functional studies) to establish such effect.[37] Therefore, the clinicians should consider the variants of uncertain significance in risk stratification.[37]

To the best of our knowledge, the current study is the first national and regional report analysing cardiomyopathy-associated genes in paediatric idiopathic primary dilated cardiomyopathy and left ventricular noncompaction patients using next-generation sequencing. Genetics underlying cardiomyopathy might be different in Egyptian patients. Genome-wide tests (i.e., exome/genome sequencing) might be more suitable than a targeted testing approach, to improve our understanding of the genetics and management of paediatric cardiomyopathy among Egyptian patients. Molecular characterisation of cardiomyopathies in different ethnic populations will allow global comparative studies that could result in understanding the pathophysiology and heterogeneity of cardiomyopathies.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S1047951121002055

Acknowledgements. We thank the patients and their families for participating in this study. The authors would like to thank Seidman Lab team at Harvard Medical School (Professors Christine Seidman, MD, and Jonathan Seidman, Barbara McDonough, and Steven DePalma, PhD) for their help in conducting this study. A.H. was supported by The National Heart, Lung, and Blood Institute (NHLBI K8HL150284), American Heart Association, and the Saving Tiny Hearts Society.

Financial support. This work was supported by the Science and Technology Development Fund (Centers of Excellence grants, grant number 5288), Cairo, Egypt. The sponsor did not have any role in study design, data collection, analysis, or preparation of the manuscript.

Conflict of interest. None.

Ethical standards. The study was approved by the ethical committee of the Faculty of Medicine, Cairo University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. An informed consent was obtained from the parents.

References

1. Choudhry S, Puri K, Denfield SW. An update on pediatric cardiomyopathy. Curr Treat Options Cardiovasc Med 2019; 21: 36.

2. Lipshultz SE, Law YM, Asante-Korang A, et al. cardiomyopathy in children: classification and diagnosis: a scientific statement from the American Heart Association. Circulation 2019; 140: E9–E68.
3. Merlo M, Pivetta A, Pinamonti B, et al. Long-term prognostic impact of therapeutic strategies in patients with idiopathic dilated cardiomyopathy: changing mortality over the last 30 years. Eur J Heart Fail 2014; 16: 317–324.
4. Elmasry OA, Kamel TB, El-Feki NF. Pediatric cardiomyopathies over the last decade: a retrospective observational epidemiology study in a tertiary institute, Egypt. J Egypt Public Health Assoc 2011; 86: 63–67.
5. Bakeet M, Montaser MM, Allam AA, Gamal R. Childhood cardiomyopathies: a study in tertiary care hospital in Upper Egypt. Electron Physician 2016; 8: 3164–3169.
6. Adadi N, Radi FZ, Lahrouchi N, et al. Inherited dilated cardiomyopathy in a large moroccan family caused by LMNA mutation. Anatol J Cardiol 2018; 20: 65–68.
7. Yotti R, Seidman CE, Seidman JG. Advances in the genetic basis and pathogenesis of sarcomere cardiomyopathies. Annu Rev Genomics Hum Genet 2019; 20: 129–153.
8. Hänselmann A, Veltmann C, Bauersachs J, Berliner D. Dilated cardiomyopathies and non-compaction cardiomyopathy. Herz 2020; 45: 212–220.
9. Hershberger RE, Hedges DJ, Morales A. Dilated cardiomyopathy: the complexity of a diverse genetic architecture. Nat Rev Cardiol 2013; 10: 531.
10. Quiat D, Witkowski L, Zouk H, Daly KP, Roberts AE. Retrospective analysis of clinical genetic testing in pediatric primary dilated cardiomyopathy: testing outcomes and the effects of variant reclassification. J Am Heart Assoc 2020; 9: e016195.
11. Dong X, Fan P, Tian T, et al. Recent advancements in the molecular genetics of left ventricular noncompaction cardiomyopathy. Clin Chim Acta 2017; 465: 40–44.
12. Pignatelli RH, McMahon CJ, Dreyer WJ, et al. Clinical characterization of left ventricular noncompaction in children: a relatively common form of cardiomyopathy. Circulation 2003; 108: 2672–2678.
13. Oechslin EN, Attenhofer Jost CH, Rojas JR, Kaufmann PA, Jenni R. Long-term follow-up of 34 adults with isolated left ventricular noncompaction: a distinct cardiomyopathy with poor prognosis. J Am Coll Cardiol 2000; 36: 493–500.
14. Shawky RM, Elsayed NS, Ibrahim DS, Seifeldin NS. Profile of genetic disorders prevalent in northeast region of Cairo, Egypt. Egypt J Med Hum Genet 2012; 13: 45–62.
15. Sobeih AA, El-Saiedi SA, Abdel Khalek NS, Attia SA, Hanna BM. Parameters affecting outcome of paediatric cardiomyopathies in the intensive care unit: experience of an Egyptian tertiary centre over 7 years. Libyan J Med 2020; 15: 1822073.
16. Sabater-Molina M, Pérez-Sánchez I, Hernández del Rincón JP, Gimeno JR. Genetics of hypertrophic cardiomyopathy: a review of current state. Clin Genet 2018; 93: 3–14.
17. Darwish RK, Haghighi A, Seliem ZS, et al. Genetic study of pediatric hypertrophic cardiomyopathy in Egypt. Cardiol Young 2020; 30: 1910–1916.
18. Miller EM, Hinton RB, Czosek R, et al. Genetic testing in pediatric left ventricular noncompaction. Circ Cardiovasc Genet 2017; 10: e001735.
19. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA guideline for the management of heart failure: executive summary: a report of the American college of cardiology foundation/American Heart Association task force on practice guidelines. Circulation 2013; 128: 1810–1852.
20. Richardson P, McKenna RW, Bristow M, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task force on the definition and classification of cardiomyopathies. Circulation 1996; 93: 841–842.
21. Jenni R, Oechslin E, Schneider J, Attenhofer Jost C, Kaufmann PA. Echocardiographic and pathoanatomical characteristics of isolated left ventricular non-compaction: a step towards classification as a distinct cardiomyopathy. Heart 2001; 86: 666–671.
22. Poplin R, Ruano-Rubio V, DePristo MA, et al. Scaling accurate genetic variant discovery to tens of thousands of samples. bioRxiv 2018: 201178. https://www.biorxiv.org/content/10.1101/201178v3.article-metrics
23. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010; 38: e164.
24. Aguib Y, Allouba M, Afify A, et al. The Egyptian Collaborative Cardiac Genomics (ECCO-GEN) Project: defining a healthy volunteer cohort. NPJ Genomic Med 2020; 5: 46.
25. Scott EM, Halees A, Itan Y, et al. Characterization of greater middle eastern genetic variation for enhanced disease gene discovery. Nat Genet 2016; 48: 1071–1079.
26. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405–424.
27. Doolan A, Tebo M, Ingles J, et al. Cardiac troponin I mutations in Australian families with hypertrophic cardiomyopathy: clinical, genetic and functional consequences. J Mol Cell Cardiol 2005; 38: 387–393.
28. Van Driest SL, Ellsworth EG, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ. Prevalence and spectrum of thin filament mutations in an outpatient referral population with hypertrophic cardiomyopathy. Circulation 2003; 108: 445–451.
29. Walsh R, Thomson KL, Ware JS, et al. Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. Genet Med 2017; 19: 192–203.
30. Nguyen S, Siu R, Dewey S, Cui Z, Gomes AV. Amino acid changes at arginine 204 of Troponin I result in increased calcium sensitivity of force development. Front Physiol 2016; 15: 520.
31. Gambarin FI, Tagliani M, Arbustini E. Pure restrictive cardiomyopathy associated with cardiac troponin I gene mutation: mismatch between the lack of hypertrophy and the presence of disarray. Heart 2008; 94: 1257.
32. Lu C, Wu W, Liu F, et al. Molecular analysis of inherited cardiomyopathy using next generation semiconductor sequencing technologies. J Transl Med 2018; 16: 241.
33. Wang C, Hata Y, Hirono K, et al. A wide and specific spectrum of genetic variants and genotype-phenotype correlations revealed by next-generation sequencing in patients with left ventricular noncompaction. J Am Heart Assoc 2017; 6: e006210.
34. Dellefave LM, Pytel P, Mewborn S, et al. Sarcomere mutations in cardiomyopathy with left ventricular hypertrabeculation. Circ Cardiovasc Genet 2009; 2: 442–449.
35. Farnaes L, Hildreth A, Sweeney NM, et al. Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization. NPJ genomic Med 2018; 3: 10.
36. Coppini R, Ho CY, Ashley E, et al. Clinical phenotype and outcome of hypertrophic cardiomyopathy associated with thin-filament gene mutations. J Am Coll Cardiol 2014; 64: 2589–2600.
37. Burstein DS, Gaynor JW, Griffis H, et al. Genetic variant burden and adverse outcomes in pediatric cardiomyopathy. Pediatr Res 2020. https://www.nature.com/articles/s41390-020-1101-5
38. Gigli M, Begay RL, Morea G, et al. A review of the giant protein titin in clinical molecular diagnostics of cardiomyopathies. Front Cardiovasc Med 2016; 3: 21.
39. Merlo M, Sinagra G, Carniel E, et al. Poor prognosis of rare sarcomeric gene variants in patients with dilated cardiomyopathy. Clin Transl Sci 2013; 6: 424–428.
40. Roberts AM, Ware JS, Herman DS, et al. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. Sci Transl Med 2015; 7: 270ra6.
41. Herman DS, Lam L, Taylor MRG, et al. Truncations of titin causing dilated cardiomyopathy. N Engl J Med 2012; 366: 619–628.
42. Towbin JA. Pediatric primary dilated cardiomyopathy gene testing and variant reclassification: does it matter? J Am Hear Assoc 2020; 9: e016910.

https://doi.org/10.1017/S1047951121002055 Published online by Cambridge University Press