Cardiology in the Young

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Genetic study of pediatric hypertrophic cardiomyopathy in Egypt

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Abstract

Paediatric cardiomyopathy is a progressive and often lethal disorder and the most common cause of heart failure in children. Despite their severe outcomes, their genetic etiology is still poorly characterised. The current study aimed at uncovering the genetic background of idiopathic primary hypertrophic cardiomyopathy in a cohort of Egyptian children using targeted next-generation sequencing. The study included 24 patients (15 males and 9 females) presented to the cardiomyopathy clinic of Cairo University Children's Hospital with a median age of 2.75 (0.5–14) years. Consanguinity was positive in 62.5% of patients. A family history of hypertrophic cardiomyopathy was present in 20.8% of patients. Ten rare variants were detected in eight patients; two pathogenic variants (8.3%) in *MBPC3* and *MYH7*, and eight variants of uncertain significance in *MYBPC3*, *TTN*, *VCL*, *MYL2*, *CSRP3*, and *RBM20*.

Here, we report on the first national study in Egypt that analysed sarcomeric and non-sarcomeric variants in a cohort of idiopathic paediatric hypertrophic cardiomyopathy patients using next-generation sequencing. The current pilot study suggests that paediatric hypertrophic cardiomyopathy in Egypt might have a particular genetic background, especially with the high burden of consanguinity. Including the genetic testing in the routine diagnostic service is important for a better understanding of the pathophysiology of the disease, proper patient management, and at-risk detection. Genome-wide tests (whole exome/genome sequencing) might be better than the targeted sequencing approach to test primary hypertrophic cardiomyopathy patients in addition to its ability for the identification of novel genetic causes.

Hypertrophic cardiomyopathy (OMIM 192600) is the most frequent genetic disease of the heart in adult.[1] Hypertrophic cardiomyopathy is characterised clinically by hypertrophy of the left ventricle unexplained by secondary causes, with preserved systolic function and impaired relaxation.[2] The cumulative morbidity and mortality of hypertrophic cardiomyopathy are substantial.[3]

The prevalence of hypertrophic cardiomyopathy was estimated to be 0.16 to 0.29% (~1:625–1:344 individuals).[4–7] It comprises about 30–40% of cardiomyopathies in children.[8] Paediatric cardiomyopathy is rare with an incidence of 1 case per 100,000 person-years in children <20 years of age.[9–11]

Cardiomyopathy is endemic in Africa and constitutes a big challenge because of its great prevalence, the difficult diagnosis often requiring specialised diagnostics that are not feasible in low- and middle-income countries, and the unavailability of effective interventions (e.g., heart transplantation).[12] Data on the magnitude of cardiomyopathy as a health problem in Egypt are scarce due to the lack of a national registry.[13]

In general, the high prevalence of genetic disorders became a great public health problem in Egypt.[14] This is mainly because of the poor access to the genetic services due to the limited resources, the lack of trained medical genetists,[15] and the cultural and financial constraints impeding the implementation of preventive genetic programs.[16] Another factor is the high rate of consanguinity. It is estimated that prevalence of consanguinity in different parts of Egypt ranges from 29 to 70%,[17] up to 86% of consanguineous marriages are between the first cousins.[14] This high rate of consanguinity rate led to the high birth prevalence of recessive disorders,[18] the appearance of new *autosomal recessive* diseases, the homozygosity *in autosomal dominant* disorders, and the higher risk of infant and child mortality.[14] In an Egyptian study of 50 children

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having cardiomyopathy at Sohag University Hospital, Egypt, consanguinity was positive in 64% of patients, and family history was present in 22% patients.[19] Despite that autosomal dominant inheritance pattern in hypertrophic cardiomyopathy is agreed upon in most studies, in a study by El-Saiedi et al which involved 10 familial hypertrophic cardiomyopathy Egyptian children, autosomal recessive inheritance was the most common mode of inheritance.[8]

Understanding the underlying mechanisms of cardiomyopathy in specific populations is necessary to develop careful strategies for its treatment and prevention. The diagnostic work-up of paediatric hypertrophy cardiomyopathy is complex and may necessitate an interdisciplinary approach to unravel the underlying cause.[20] The rapid advancement of personalised and genomic medicine offered a great opportunity in this concern.[21] Genetic testing is particularly valuable for the diagnosis and classification of paediatric hypertrophy cardiomyopathy.[22]

Many of published studies on paediatric hypertrophic cardiomyopathy have used Sanger sequencing.[23–25] Moreover, these studies were focussed on patients from North America and Europe and tested only sarcomeric genes.[26] Only 18 studies reported on the hypertrophic cardiomyopathy genetics in Africa; 15 in South Africa, and three in Egypt, Tunisia, and Morocco.[27] Only three of those studies employed the next-generation sequencing technology.[28–30]

At Cairo University Children's Hospital, the biggest tertiary hospital in Egypt, the prevalence of paediatric hypertrophic cardiomyopathy was 50/10,000 cardiac cases presenting to the hospital in 1 year.[8] In a retrospective study at the same hospital (2004–2016), hypertrophic cardiomyopathy accounted for 260/1282 (20%) of cardiomyopathy cases[31] and they had a relatively worse prognosis than the previously reported in Western and Asian patients.[32] Despite the high number of hypertrophic cardiomyopathy patients presenting to the cardiomyopathy clinic of the hospital,[8] genetic testing has not been routinely performed due to the limited resources. To elucidate the genetic background of hypertrophic cardiomyopathy in Egyptian children, we established a next-generation sequencing program at Cairo University Children's Hospital as a nationally-funded project. As a pilot study, 24 paediatric hypertrophic cardiomyopathy patients were tested using a next-generation targeted sequencing panel.

Materials and methods

The current study tested 24 unrelated patients aged <16 years who presented to the Paediatric Cardiomyopathy Clinic of Cairo University Children's Hospital with primary idiopathic hypertrophic cardiomyopathy. Exclusion criteria included patients older than 16 years, infants of diabetic mothers, patients with secondary causes such as systemic hypertension, aortic stenosis (valvular and sub-valvular), infiltrative cardiomyopathies, storage disorders, and other metabolic causes or intake of drugs causing cardiac hypertrophy. Syndromic patients and patients with extracardiac manifestations were also excluded. Included cases were subjected to the following clinical and genetic evaluations:

1. **Clinical workup**: A) A complete medical history including antenatal, natal histories, any maternal chronic illness, exposure to drug intake, and history of cardiac symptoms especially those favouring the diagnosis of cardiomyopathy, such as dyspnea, palpitation, recurrent chest infections, and syncopal attacks, B) A full family history along with a three-generation pedigree that includes information on consanguinity, similar conditions in the same family or other genetic diseases. Familial hypertrophic cardiomyopathy was considered if at least one of the family members of the proband was diagnosed with the disease,[33] C) Anthropometric measurements (e.g., weight, height, and head circumference), D) Full clinical examination, E) Measurement of the blood pressure to exclude systemic hypertension, F) Plain chest X-ray, and G) Electrocardiogram and echocardiography (M-mode and Doppler). Electrocardiogram and echocardiography were done for the cases and parents as well. Echocardiographic parameters included left ventricular end-systolic dimension, left ventricular end-diastolic dimension, maximum left ventricular wall thickness, interventricular septal thickness, left ventricular fractional shortening, left ventricular ejection fraction, and left ventricular outflow tract pressure gradient. Patterns of hypertrophy were also reported.[34] The left ventricular diastolic wall thickness > 2 SD from the predicted mean was the base of diagnosis of hypertrophic cardiomyopathy (z-score > 2, and z-score is defined as the number of SD from the body surface area adjusted mean in the normal population).[35] Hypertrophic obstructive cardiomyopathy was considered if the left ventricular outflow tract pressure gradient is more than 30 mmHg,[36] H) Heart failure was assessed according to ROSS/NYHA functional classification,[37] I) Other investigations were performed as appropriate, for example, for the exclusion of inborn errors of metabolism presenting with cardiomyopathy, laboratory assessment included routine lab investigations (complete blood picture, liver and kidney function tests, electrolytes, creatine kinase, and blood glucose), and specialised lab investigations (extended metabolic screen for the exclusion of amino acid, fatty acid and organic acid disorders, and enzymatic assays for lysosomal and storage diseases).
2. **Genetic Testing**. Targeted enrichment next-generation sequencing was performed on Illumina MiSeq system using TruSight Cardio panel (Illumina, San Diego, CA, USA).[38] This panel contains 174 genes with known associations to 17 inherited cardiac conditions.

DNA Extraction and quantification. Genomic DNA was extracted using a DNA extraction kit (NucleoSpin Blood QuickPure, Macherey Nagel GmbH & Co., Germany). The DNA was quantified using Qubit® dsDNA HS assay kit (Invitrogen, Grand Island, NY, USA) and the Qubit 2.0 Fluorimeter (Thermo Fisher Scientific, CA, USA).

Library preparation. Target regions were captured by in-solution hybridisation according to the manufacturer protocol. The targeted regions were sequenced using the MiSeq platform, generating two million of 150 bp paired-end reads for each sample (Q30 ≥ 90%).

Bioinformatic data analysis and variant classification. The MiSeq Reporter software installed on-instrument was used for demultiplexing the data and raw FASTQ file generation. Bam files were made using the Burrows–Wheeler Aligner[39] which aligned the reads against the human reference genome GRCh37/hg19. Variants were called using the Genome Analysis Toolkit[40] and variant call format files were created. A run is called accepted if at least 95% of the target regions were covered with an average depth of coverage 100X. Integrated Genome Viewer 2.4 was used to visually inspect sequence reads and variant positions.[41] A variant was accepted if it had a quality score > 100 and covered by at least 50 reads. The variant call format files were then annotated using in-house developed scripts based on the ANNOVAR knowledge

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database.[42] The annotation included information about the physical position of the variant in the genome and the protein using SnpEff (v4.3 T)[43] (http://snpeff.sourceforge.net/) and the minor allele frequency of variants in public databases such as Genome Aggregation Database (http://gnomad.broadinstitute.org/), 1000 genome, and dbSNP (V138) (www.ncbi.nlm.nih.gov/SNP/). The effect of the variants on the protein structure was predicted using bioinformatics' algorithms such as Sorting Tolerant From Intolerant (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and Combined Annotation Dependent Depletion (CADD).[44] Clinical annotation was performed using Online Mendelian Inheritance in Man (https://omim.org), Human Gene Mutation Database (HGMD Professional 2019.4; http://www.biobase-international.com/), and ClinVar[45] (http://www.ncbi.nlm.nih.gov/clinvar/).

The variants were filtered to exclude non-relevant variants (e.g., synonymous and intronic variants). Variants with minor allele frequency >0.01% in population databases were considered frequent and excluded. Exonic variants predicted to be tolerant/benign by Sift/Polyphen/CADD were also excluded. The remaining variants were evaluated by investigating their records in Online Mendelian Inheritance in Man, Human Gene Mutation Database, and ClinVar. The panel included 15 recessive genes related to cardiomyopathy (*ALMS1, COX15, DMD, FKRP, FKTN, FXN, GAA, GATAD1, HADHA, LAMA2, SCO2, SDHA, SGCB, SGCD, and SGCG)*. Online Mendelian Inheritance in Man was used to check the inheritance model of the gene, and a variant not matching the model was excluded. Specifically, a heterozygous variant in a gene whose inheritance model was autosomal-recessive was considered a carrier and not considered the disease cause. The pathogenicity of the variant was evaluated using the Human Gene Mutation Database and ClinVar, any variant classified as benign was also excluded. The remaining variants were examined by extensive literature search to evaluate their contribution to the disease. Finally, the variants were classified according to the American College of Medical Genetics and Genomics guidelines[46] as a pathogenic, likely pathogenic, variant of uncertain significance, benign or likely benign.

The identified variants were confirmed by Sanger sequencing using the ABI 3730XL DNA analyzer (Applied Biosystems, Foster, CA, USA).

Results

The current study included 24 hypertrophic cardiomyopathy patients (15 males and 9 females) presented to Cairo University Children's Hospital. The patients' age ranged from 0.5 to 14 years with a median of 2.75 years. The initial age of presentation was below 2 years in 18/24 (75%) of patients. Consanguinity was positive in 15/24 (62.5%) of patients. Positive family history was present in 5/24 (20.8%) of patients. The main presenting symptoms were dyspnea (83.3%) followed by recurrent chest infections (16.6%). Asymmetrical septal hypertrophy was the most common phenotype (58.3%). Left ventricle outflow tract obstruction was present in 7/24 (29%) of patients. The electrocardiogram of all patients showed a varying degree of sinus tachycardia and high voltage left ventricular preponderance and no one had arrhythmia. Demographic, clinical, and echocardiography data of the patients are summarised in Table 1. Full clinical data can be found in supplementary Table 1.

Among the 24 patients, 10 rare variants were detected in 8 patients. Per the American College of Medical Genetics and Genomics guidelines,[46] we classified *MYBPC3*:p.R495G, *MYH7*:p.R403Q as "Pathogenic" explaining only 2/24 (8.3%) of patients. Four other variants, that were reported in ClinVar: *MYL2*:p.Q38R,

Table 1. Demographic, clinical and echocardiographic characteristics of the studied group

All patients (n = 24)	
Age at diagnosis (y)	2.25 (0.5–14)
Male/female	14/9 (36/56)
Wt (kg)	10.6 (3–60)
Ht (cm)	84.5 (48–159)
Time since onset (y)	2 (0.25–10)
Course (pr/st)	3/22 (12/88)
Consanguinity	15 (62.5)
Family history of HCM	5 (20.8)
Symptoms/signs	
Dyspnea	20 (83.3)
Recurrent chest infection	4 (16.6)
Syncope	2 (8.3)
Tachycardia	2 (8.3)
Hepatomegaly	6 (25)
NYHA/ROSS classification	
Yes I	7 (29.1)
II	6 (25)
No	11 (45.8)
Echocardiography	
LVEDD (mm)	25.5 (12–66.1)
LVEDD z score	−1.38 (−4.8–7.7)
LVESD (mm)	14 (6–59.5)
LVESD z score	−1.83 (−5.6–12.2)
IVS (mm)	8.5 (1.1–33)
IVS z score	7.29 (1.9–29.3)
LVWT (mm)	7 (0.9–21)
LVWT z score	2.4 (−0.8–27)
FS (%)	43 (13–71)
EF (%)	79.5 (26–95)
Patients with LVOTO (%)	7 (29.1)
Pattern of LVH	
ASH	14 (58.3)
Concentric	5 (20.8)
Focal	1 (4.1)
Mid cavitary	2 (8.4)
Localized	1 (4.2)
Sub-aortic	1 (4.2)

Data are presented as n (%) or Median (interquartile range). ASH: asymmetric septal hypertrophy, HCM: Hypertrophic Cardiomyopathy, IVS: Interventricular septal thickness, LVEDD: Left ventricular end-diastolic dimension, LVESD: Left ventricular end-systolic dimension, LVH: Left ventricular hypertrophy, LVOTO: left ventricular outflow tract obstruction, LVWT: Left ventricular wall thickness, NYHA: New York Heart Association, Pr: Progressive, St: Stationary, Y: Year

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Table 2. Variants identified in eight HCM patients

Pt No.	GT	Gene ID	Variant	rs ID	gnomAD-AF	Poly-Phen2	CADD	Classification	ClinVar ID
199	Het	*MYBPC3*	NM_000256:c.1483C>G (p.R495G)-exon 16	rs397515905	0.000004	0.909 (D)	25.4	Pathogenic	42537
	Het	*TTN*	NM_001267550:c.34612+1G>T-exon 150	rs577363824	0.00001	.	25.9	VUS	404922
235	Het	*MYH7*	NM_000257:c.1208G>A (p.R403Q)-exon 13	rs121913624	–	1 (D)	33	Pathogenic	14087
214	Hom	*MYL2*	NM_000432:c.113A>G (p.Q38R)-exon 3	rs730880947	–	0.823 (P)	27.1	VUS	81429
	Het	*TTN*	NM_003319:c.76289T>C (p.L25430P)-exon 186	rs373479287	0.00002	1 (D)	22.2	VUS	–
202	Het	*MYBPC3*	NM_000256:c.225C>A (p.D75E)-exon 2	rs1395765226	0.000†	1 (D)	23.5	VUS	–
217	Het	*VCL*	NM_003373:c.2046A>T (p.L682F)-exon 15	rs565398652	0.00004	0.997 (D)	23.3	VUS	166551
232	Het	*TTN*	NM_003319:c.48053C>T (p.A16018V)-exon 154	–	–	0.989 (D)	15.3	VUS	–
247	Het	*CSRP3*	NM_003476:c.365G>A (p.R122Q)-exon 5	rs193922667	0.00001	1 (D)	35	VUS	35966
256	Het	*RBM20*	NM_001134363:c.3035A>G (p.E1012G)-exon 11	–	–	0.988 (D)	29.5	VUS	–

D: damaging, GT: Genotype, Het: heterozygous, Hom: homozygous, P: probably damaging, Pt No.: Patient Number, VUS: variant of unknown significance.
*Allele Count is zero (i.e., no high-confidence genotype)

CSRP3:p.R122Q, *VCL*:p.L682F, and *TTN*:c.34612+1G>T, were classified as variants of uncertain significance. We also identified four rare variants (*TTN*:p.L25430P, *TTN*:p.A16018V, *MYBPC3*:p.D75E; *RBM20*:p.E1012G) that have not been previously reported in hypertrophic cardiomyopathy patients. These variants were also classified as variants of uncertain significance per the American College of Medical Genetics and Genomics guidelines (Table 2).[46]

The pathogenic variant *MYBPC3*:p.R495G was detected in a 14-year-old male (patient 191) who presented at the age of 4 years with low cardiac output symptoms mainly dyspnea on exertion. Echocardiography examination revealed hypertrophic obstructive cardiomyopathy. The patient was born to consanguineous parents who were examined by echocardiography and were healthy. The proband had a younger brother who presented at the age of 3 years with the same symptoms and was diagnosed with hypertrophic obstructive cardiomyopathy as well. This variant had been previously reported in children (sporadic[23] and familial[47]), in adult patients with overt phenotype[48] and non-affected family members.[49]

The pathogenic variant *MYH7*:p.R403Q was detected in a 13-year-old male (patient 235) presented at the age of 3 years with dyspnea on exertion and tachycardia. Electrocardiogram was normal and echocardiography was consistent with hypertrophic cardiomyopathy. The patient was born to non-consanguineous parents. The patient's father had hypertrophic cardiomyopathy and died at the age of 40 and he had two uncles died with hypertrophic cardiomyopathy as well. This variant had been reported many times in association with hypertrophic cardiomyopathy, either familial[50–52] or sporadic[53] in both children[24] and adults.[54] Some of the previously reported patients presented with early-onset, severe phenotype, or premature death.[24,50] Many functional studies using animal models[55,56] and in vitro testing supported the functional impact of this variant.[57] This variant has also been reported in a patient with left ventricular non-compaction (OMIM 300183)[58] and another patient with hypertrophic cardiomyopathy, left ventricular non-compaction, and coronary fistulae.[59]

Discussion

Paediatric cardiomyopathies are progressive and often lethal disorders and the most common cause of heart failure in children.[60] Despite their severe outcomes, their genetic etiology is still poorly characterised.[60] The massively parallel sequencing capabilities of next-generation sequencing made it possible to study many or even all genes simultaneously at an affordable cost and time.[61] A guideline by the Heart Failure Society of America and the American College of Medical Genetics and Genomics recommended genetic testing for cardiomyopathies using multi-gene panel testing. The most important indication for genetic testing in hypertrophic cardiomyopathy patients is the identification of the causative variant that enables screening the relatives who are at risk, early diagnosis, and identifying young mutation carriers many years before clinical disease onset. This enables clinical surveillance of mutation carriers and prevents unnecessary follow-up of non-carriers.[62]

Sarcomeric variants are the most common cause of hypertrophic cardiomyopathy in children.[24] Mutations in *MYH7* and *MYBPC3* are the most common cause of paediatric hypertrophic cardiomyopathy, which is similar to the adult hypertrophic cardiomyopathy.[23,63] Mutations in other sarcomere genes, *TNNT2, TNNI3, TPM1, ACTC1, MYL2*, and *MYL3*, can also cause hypertrophic cardiomyopathy in children.[64] Non-sarcomeric genetic variants had been identified in children with ventricular hypertrophy and malformation syndromes, inborn errors of metabolism, or neuromuscular disorders.[65]

In the current study, cardiomyopathy associated genes were analysed in 24 unrelated paediatric hypertrophic cardiomyopathy patients. Ten variants were detected in eight patients; two variants were pathogenic (*MYBPC3* p.R495G and *MYH7*:p.R403Q) explaining only 8.3% of patients, and eight variants of uncertain significance in *MYBPC3, MYL2, RBM20, CSRP3, VCL*, and *TTN*. Those variants of uncertain significance are rare and predicted in Silico to be damaging; however, further studies including functional studies and segregation analyses remain to be done to confirm their potential role in the pathogenesis of hypertrophic cardiomyopathy.

In this study, 22 (91.6%) patients remained genetically unsolved, of which 3 had a positive family history of hypertrophic cardiomyopathy. Studies from the National Heart, Lung, and Blood Institute-funded Paediatric Cardiomyopathy Registry have shown that causes are established in very few children with cardiomyopathy, yet genetic causes are likely to be present in most.[66] The positive detection rate (8.3% with a pathogenic or likely pathogenic variant) reported in the current study is lower than that (26–39%) of reported in other populations.[60,67,68] The differences in the total

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positive rate among the different population 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 including different age groups[69] as shown in Supplementary Table 2. The lower positive genetic detection rate in the studied group suggests that the genetics of hypertrophic cardiomyopathy in Egyptian patients might be different, particularly with the high rate of consanguinity.

Studies had investigated a few hypertrophic cardiomyopathy genes (*MYBPC3, MYH7*, and *TNNT2*) in Egyptian patients[70,71] using different methodologies from denaturing high-performance liquid chromatography/Sanger technologies to targeted next-generation sequencing panels. These studies tested patients with a wide range of age of onset (2–70 years with 53% <40 years), whereas the current study exclusively investigated children <16 years. This is the first national study analysing sarcomeric and non-sarcomeric mutations in a cohort of Egyptian paediatric hypertrophic cardiomyopathy patients using next-generation sequencing. This work had a number of limitations including the small sample size that limited statistical analysis, lack of segregation analysis, and functional studies. In addition, although there is no sequencing database of Egyptian population, we used gnomAD, which is the largest publicly available dataset for population-based allele frequencies, for our analyses.

In Egypt, with the high burden of consanguinity and inherited diseases, including genetic testing in the routine diagnostic service is important. Currently, in Egypt, even the major sarcomere genes are not routinely tested in cardiomyopathy patients. One of the most important steps to promote the practice of genomic medicine is improving the knowledge of physicians about the benefits of proper genetic testing for better patient outcomes through timely therapeutic strategies and early intervention in at-risk cases.

Conclusion

Similar to adult hypertrophic cardiomyopathy, mutations in *MYPC3* and *MYH7* are the major cause of hypertrophic cardiomyopathy in children. Next-generation sequencing is an important tool for uncovering the genetic background of idiopathic paediatric hypertrophic cardiomyopathy. Our data suggests a hypothesis that the genetics of paediatric hypertrophic cardiomyopathy might be different in Egyptian patients. Genome-wide tests (i.e., whole 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 children. Segregation analysis, functional studies, and the development of a large Egyptian control database are highly needed for genetic testing in Egypt, especially to confirm the pathogenicity of the potential variants of uncertain significance.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S1047951120003157

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional committees (the ethical committee of the Faculty of Medicine, Cairo University).

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