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ORIGINAL ARTICLE

Targeted exome analysis of Russian patients with hypertrophic cardiomyopathy

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Abstract

Background: Hypertrophic cardiomyopathy (HCM), described as the presence of hypertrophy of left ventricular, is the most prevalent heritable cardiovascular disease with predominantly an autosomal dominant type of inheritance. However, pathogenic alleles are not identified in at least 25% of patients with HCM, and the spectrum of pathogenic variants that contribute to the development of HCM in Russia has not been fully described. Therefore, the goal of our study was to identify genetic variants associated with the etiopathogenesis of HCM in Russian patients.

Methods: The study cohort included 98 unrelated adult patients with HCM. We performed targeted exome sequencing, an analysis using various algorithms for prediction of the impact of variants on protein structure and the prediction of pathogenicity using ACMG Guidelines.

Results: The frequency of pathogenic and likely pathogenic variants in all HCM-related genes was 8% in our patients. We also identified 20 variants of uncertain significance in all HCM-related genes.

Conclusions: The prevalence of individual pathogenic variants in HCM-related genes in Russian population appears to be lower than in general European population, which could be explained by ethnic features of Russian population, age characteristics of our sample, or unidentified pathogenic variants in genes previously not linked with HCM.

KEYWORDS

exome, genetics, hypertrophic cardiomyopathy, next generation sequencing, pathogenic variants

1 | INTRODUCTION

Hypertrophic cardiomyopathy (HCM), described as the presence of idiopathic left ventricular hypertrophy, is the most prevalent heritable cardiovascular disease and affects more than 1 in 500 individuals (Semsarian et al., 2015). This condition in some cases leads to heart failure, sudden cardiac death (SCD), and atrial fibrillation followed by embolic stroke (Cecconi et al., 2016; Maron & Maron, 2013).

Hypertrophic cardiomyopathy is predominantly a genetically caused disorder with an autosomal dominant type of inheritance (Greaves et al., 1987; Ho et al., 2015; Maron & Maron, 2013; Maron et al., 2012). Rare autosomal recessive and X-linked types of inheritance have also been described (Branzi et al., 1985; Hagen et al., 2015; Hartmannova et al., 2013; Santorelli et al., 1999) and may be a phenocopy condition (Marian, 2016). Moreover, numerous sporadic cases associated with de novo pathogenic variants have also been reported (Maron et al., 2012; Watkins et al., 1995).

Currently, HCM is associated with over two dozens of different mutant genes, which predominantly encode thick and thin myofilament (TTm) proteins of the sarcomere. Disease-causing pathogenic variants in myosin heavy chain 7 (*MYH7*) and myosin binding protein C, cardiac (*MYBPC3*) genes, which encode myofilament proteins, account for ~70% of more than 1,350 alleles with proven pathogenetic significance that have been identified in patients with HCM (van Velzen et al., 2018). Pathogenic variants not encoding for TTm proteins have also been detected in several patients with this condition (Arimura et al., 2009; Chiu et al., 2007; Hayashi et al., 2004; Osio et al., 2007; Siegert et al., 2011; Vasile et al., 2006).

Nevertheless, pathogenic alleles are not present in 28–40% of patients with HCM and a family history of the disease and in up to 70% of sporadic HCM cases (Alfares et al., 2015; Walsh, Buchan, et al., 2017). Conversely, up to 5% of patients with HCM carry several pathogenic variants affecting one or more gene(s) (Burns et al., 2017). Moreover, a large genetic diversity may also be associated with a large number of clinical manifestations from asymptomatic to SCD (Maron et al., 2012), even in representatives of the same family with the same pathogenic variant (Roberts et al., 2013). Some pathogenic variants demonstrate incomplete penetrance, which may depend on environmental and/or other genetic factors. Many rare pathogenic variants with low-to-moderate penetrance are detected in patients with sporadic form of HCM and in small families with this disorder. Thus, it is very difficult to establish the role of such variants in the etiopathogenesis of HCM (Marian & Braunwald, 2017).

In this context, it is very important to study the genetic landscape of HCM in diverse populations. Moreover, apart from several reports on single pathogenic variants in small samples of Russian patients (Glotov et al., 2015; Kostareva et al., 2006; Savostyanov et al., 2017; Seleznev et al., 2005), the spectrum of genes and pathogenic variants in them that contribute to the development of this disease in our population has not been described on a large scale. Conducting such studies will complement the knowledge on the range of pathogenic variants and genes involved in the development of HCM and allow the expansion of our understanding of the mechanisms of pathogenesis of HCM and the functioning of the cardiovascular system as a whole. Therefore, the goal of our study was to identify genetic variants associated with the etiopathogenesis of HCM in patients from Russia – both known pathogenic variants in genes responsible for the development of the disease and new likely pathogenic variants with the possible identification of new genes associated with the development of HCM.

2 | METHODS

2.1 | Ethical compliance

The study was approved by the Ethics Committees of RNRMU and Institute of Molecular Genetics.

2.2 | Patients

Studied cohort included 98 unrelated adult patients with HCM. The subjects were all Russians from the Moscow region. Patients were selected and investigated according to the European diagnostic criteria for familial HCM (interventricular septal thickness (IVS) ≥15 mm in the absence of other known causes of hypertrophy) (Elliott et al., 2014) by Krylova N.S. at Cardiological Department of City Clinical Hospital No. 52 of the Moscow City Health Department). The average age of the patients at the time of enrollment in the study was 58.59 ± 14.66 years, and the sex ratio was 39/59 (M/F). 35 patients had an older relative with SCD and/or HCM (24 patients had only an older relative with SCD, 6 patients had only an older relative with HCM, 5 patients had both). Clinical characteristics are presented in Table 1. Written informed consent was obtained from all participating patients and families according to the Declaration of Helsinki.

2.3 | DNA preparation and sequencing

Genomic DNA was obtained from leukocytes using a Quick-DNA Miniprep Kit (Zymo Research), according

TABLE 1 Clinical characteristics of the patients enrolled in the study.

	N (male)
Family history of cardiomyopathy	12 (3)
Family history of sudden death	29 (9)
Clinical death	0
Use of Implantable Cardioverter Defibrillator (ICD)	2 (1)
Myoectomy	3 (1)
History of sudden cardiac arrest: myocardial infarction	0
Chest pain:	
Angina	43 (12)
Cardialgia	34 (11)
Syncope/history of syncope	5 (0)/13 (2)
Dizziness	29 (10)
Dyspnea	74 (30)
Noises in a heart	28 (12)
Palpitations	44 (15)
ECG abnormalities	64 (26)
Hypertension	78 (27)
I functional class of heart failure	7 (6)
II functional class of heart failure	77 (33)
III functional class of heart failure	17 (3)
IV functional class of heart failure	0

Abbreviation: N, number of patients.

to the manufacturer's instructions. The concentration of isolated nucleic acids was measured using a Qubit fluorometer (Invitrogen) and a Quant-iT DNA BR Assay Kit (Invitrogen), according to the manufacturer's instructions. Exome sequencing was performed using a SureSelect Focused Exome Enrichment kit (Agilent Technologies, Inc.) on an Illumina HiSeq 2500 sequencer. The sequence consisted of ~4,800 disease-associated genes and regions with an average coverage of 30 reads. Pathogenic and likely pathogenic variants were validated by Sanger sequencing. The sequences of the primers used are available upon request.

2.4 | Annotation and functional assessment

Bioinformatic analysis of obtained sequences was carried out using various bioinformatics resources. Bioinformatic sequence analysis was performed in the "R" software environment using Ensembl Variation and Ensembl Gene data (Zerbino et al., 2018). Ensembl data was accessed using the "R" BioMart package (Smedley et al., 2015). We used the GRCh38 reference assembly (Schneider et al., 2017). Genome alignment was performed with Burrows-Wheeler Alignment (BWA) tool (Li & Durbin, 2009). The genome mapping and variant calling was conducted using GATK HaplotypeCaller, as described in GATK best practices (Poplin et al., 2017). The replacement effect was classified using the Variant Effect Predictor (McLaren et al., 2016). The impact on protein structure of the identified variants was further assessed using Polyphen-2 (Polymorphism Phenotyping v2) (Adzhubei et al., 2010), SIFT (Sorting Intolerant from Tolerant) (Sim et al., 2012), REVEL (Rare Exome Variant Ensemble Learner) (Ioannidis et al., 2016), and CADD (Combined Annotation Dependent Depletion) (Rentzsch et al., 2019). The missense variants were considered "probably damaging" (or "potentially damaging") if they had a Polyphen score > 0.5 or SIFT score < 0.05 (deleterious), and CADD PHRED >20 and REVEL Score > 0.5. Only CADD algorithm was able to evaluate the deleteriousness of variants leading to frame-shift or formation of stop-codon. These variants were considered "probably damaging" (or "potentially damaging") if they had a CADD PHRED score > 20. The pathogenicity of known variants was also assessed using ClinVar database (Landrum et al., 2018). All possibly pathogenic variants were classified in accordance with ACMG Standards and Guidelines for the Interpretation of Sequence Variants (Kelly et al., 2018; Richards et al., 2015).

3 | RESULTS

It is currently believed that HCM is predominantly an autosomal dominant disease. Therefore, the first stage of our analysis included only missense, nonsense, and small indel heterozygous variants in the coding regions of genes of interest. We selected only heterozygous variants (autosomal dominant type of inheritance was assumed) with a genotyping quality more than 99 and a coverage of at least 20 reads. The analysis of the selected variants revealed that there were strong differences in the ratio between two allelic variants that were read during the sequencing. As a result, an approach for the elimination of false-positive heterozygotes was implemented at the initial stage of the primary data analysis (Shulskaya et al., 2018). The result of the formula (AD1-AD2)/DP, where DP is the approximated read depth, and AD1 and AD2 are the approximated read depth for the first and the second allele, respectively, was used as a criterion for the selection of reliable heterozygous positions. For further analysis, we selected only variants for which the result of the formula met a specific condition, i.e., it had to be in the range of -0.3 and 0.3. The approach was implemented as a Python

(version 2.7) script and described in the Supplementary Materials elsewhere (Shulskaya et al., 2018).

As the goal of our study was to identify genetic variants associated with the development of HCM in patients from Russia, both known pathogenic variants and new likely pathogenic variants with the possible identification of new genes associated with the development of HCM, for further analysis we selected only variants with MAF <0.0001 according to gnomAD, or ALFA or ExAc, if there was no data on MAF in gnomAD.

To reduce the number of candidate variants, we analyzed only 174 genes with known associations to 17 different inherited cardiac conditions according to the TruSight Cardio Sequencing Kit (Illumina) (https://emea.support.illumina.com/downloads/trusight-cardio-product-files.html, last access: 10.08.2020). Next, we used bioinformatics resources to predict the impact of variants on protein structure. The use of the Polyphen-2, SIFT, REVEL, and CADD allowed us to select 54 possibly pathogenic variants that: (a) met the CADD criteria for nonsense variants and at least two of the criteria used for missense variants and (b) were located in the coding regions of 28 genes, including the definitive genes responsible for the development of most of the cases of familial HCM (Marian & Braunwald, 2017). Further, we analyzed only variants in the genes that are associated with the development of HCM according to the OMIM database and ClinGen resource (Rehm et al., 2015) (Table S1). The data on these variants with the scores of all algorithms used are presented in Table S2.

At the second stage, an analysis of definitive HCM genes (*ACTC1*, *MYBPC3*, *MYH7*, *MYL2*, *MYL3*, *TNNI3*, *TNNT2*, *TPM1*, and *PRKAG2*), according to the ClinGen resource, was carried out, which led to the identification of several possibly pathogenic variants. Four of these variants are now considered pathogenic variants: p.Q1233X (two probands), R495Q (one proband), and p.Y847X (one proband) in the *MYBPC3* gene and p.G741R (one proband) in the *MYH7* gene (Table 2). All of them were validated by Sanger sequencing.

We also identified several likely pathogenic variants and variants of uncertain significance in the *MYH7*, *MYBPC3*, *MYL2*, *MYL3*, and *TPM1* genes (Table 3), which were classified as probably damaging all algorithms used it the study; however, they are very rare and there are few data on their clinical significance in ClinVar database and ClinGen resource. It should be noted that three variants in the *MYH7* and *MYBPC3* genes were discovered for the first time here. The change of adenine to thymine at position 23415221 in NC_000014.9 (GRCh38.p12) (also validated by Sanger sequencing) leads to the H1778L amino-acid change, which could alter the structure of the MYH7 protein significantly. A similar situation was detected for variant K1173X (NC_000011.10:g.47332676T > A (GRCh38.p12)) (Table 3 and Table S1), which may lead to the mRNA degradation by nonsense-mediated decay or synthesis of truncated MYBPC3 protein. Unfortunately, we were unable to analyze the parents and/or children of this patient. However, the novel variant K1173X, resulting in synthesis of truncated protein, could be the cause of HCM in this case.

We also identified 13 variants of uncertain significance in the moderate and limited, according to ClinGen resource, HCM genes encoding actinin alpha 2 (*ACTN2*), LIM domain binding 3 protein (*LDB3*), titin (*TTN*), myosin heavy chain 6 (*MYH6*), nexilin F-actin binding protein (*NEXN*), and vinculin (*VCL*), which are linked to the development of HCM (Table 4). All of these variants were classified as possibly damaging by the algorithms used here. However, they are extremely rare (with the exception of some variants in the *TTN* gene) and there are no or few data on their actual pathogenicity in the ClinVar database.

4 | DISCUSSION

In this study, we aimed to identify genetic variants associated with the ethiopathogenesis of HCM in patients from Russia by screening for both known pathogenic variants and new likely pathogenic variants in genes currently associated with the development of HCM.

It should be noted that we were unable to identify major pathogenic variants that may be characteristic of our patients. The frequency of pathogenic variants in the definitive HCM genes in our patients was only 5%. However, when considering all pathogenic variants (i.e., pathogenic as well as likely pathogenic variants) in definitive, moderate and limited HCM-related genes, the frequency

TABLE 2 Pathogenic variants in definitive HCM genes

Gene	Existing variation	Position in genome	Protein position	Number of probands
MYBPC3	rs397516037	NC_000011.10:g.47332189G>A	Q1233X	2
MYBPC3	rs397515974	NC_000011.10:g.47337452G>C	Y847X	1
MYBPC3	rs200411226	NC_000011.10:g.47342718C>T	R495Q	1
MYH7	rs121913632	NC_000014.9:g.23425760C>T	G741R	1

TABLE 3 Likely pathogenic variants and variants of uncertain significance in definitive HCM genes

Gene	Existing variation	Position in genome	Protein position	Number of probands
Likely pathogenic variants				
MYBPC3	rs397515905	NC_000011.10:g.47342719G>A	R495W	1
MYBPC3	rs730880711	NC_000011.10:g.47342928_47342929insG	V453X	1
MYBPC3		NC_000011.10:g.47332676T>A	K1173X	1
Variants of uncertain significance				
MYL2	rs375667565	NC_000012.12:g.110913124G>A	T125 M	1
MYH7		NC_000014.9:g.23415221T>A*	H1778L	1
MYH7	rs200303340	NC_000014.9:g.23415421C>T	C1748Y	1
MYH7	rs397516178	NC_000014.9:g.23422291C>T	R1045H	1
MYH7	rs727503278	NC_000014.9:g.23432714G>A	R143W	1
MYL3	rs730880954	NC_000003.12:g.46860799C>T	D62N	1
TPM1		NC_000015.10:g.63061778A>G	Q252R	1

TABLE 4 Variants of uncertain significance in other HCM genes

Gene	Existing variation	Position in genome	Protein position	Number of probands
ACTN2	rs397516574	NC_000001.11:g.236761033C>T	R796C	1
NEXN		NC_000001.11:g.77935997G>C	A412P	1
VCL	rs749628307	NC_000010.11:g.74074809G>A	R230H	1
LDB3	rs774815578	NC_000010.11:g.86732915C>T	P598L	1
MYH6	rs201989347	NC_000014.9:g.23387854G>A	R1477C	1
TTN		NC_000002.12:g.178538825G>C	P31361A	1
TTN		NC_000002.12:g.178553717C>A	G28122V	1
TTN	rs192360370	NC_000002.12:g.178563052G>A	R26053C	1
TTN	rs551496477	NC_000002.12:g.178563804C>T	R25802H	1
TTN	rs1214607347	NC_000002.12:g.178568461G>T	P24250T	1
TTN	rs532157196	NC_000002.12:g.178574833G>A	R22126W	1
TTN	rs371973579	NC_000002.12:g.178594422G>A	R17717C	1
TTN	rs756003188	NC_000002.12:g.178713277G>A	P8636S	1

of pathogenic variants was 8% in our patients, whereas the mutation rate in these genes worldwide has been estimated to be 30–60% on average(Burke et al., 2016). In Europe alone, the frequency of HCM causing pathogenic variants is estimated as 17–63% (average 33.5%) (Andersen et al., 2009; Berge & Leren, 2014; Brito et al., 2012; Cecconi et al., 2016; Erdmann et al., 2003; Fokstuen et al., 2008, 2011; Garcia-Castro et al., 2009; Kaski et al., 2009; Lopes et al., 2013; Millat et al., 2010; Morner et al., 2003; Richard et al., 2003; Waldmuller et al., 2011; Zeller et al., 2006). This discrepancy of our results with the data obtained on other populations could be explained by the ethnic characteristics of our sample. Moreover, the fact that our sample consists mainly of middle-aged patients without family history of the disease may be associated with unidentified pathogenic variants in genes previously not linked with HCM, which alone or in combination could result in manifestation of mild form of HCM.

It is widely recognized that HCM is caused by rare pathogenic variants. These variants are usually found in domains of genes that encode sarcomere proteins and proteins associated with this cell structure. Accordingly, the majority of pathogenic variants in our sample were identified in the sarcomere-related genes *MYH7* and *MYBPC3*, the mutation of which is causative in majority of cases of HCM with a proven genetic cause (Marian & Braunwald, 2017). Overall the frequency of these pathogenic variants (Table 2) in our sample was 5%, which was higher than that reported in various European samples (average 0.86%) (Berge & Leren, 2014; Brito et al., 2012; Cecconi et al., 2016; Christiaans et al., 2010; Ehlermann et al., 2008; Erdmann et al., 2001; Fokstuen et al., 2008,

2011; Garcia-Giustiniani et al., 2015; Helms et al., 2014; Ingles et al., 2013; Kapplinger et al., 2014; Kaski et al., 2009; Lopes et al., 2013; Millat et al., 2010; Ng et al., 2013; Niimura et al., 1998; Perrot et al., 2005; Toth et al., 2011; Weissler-Snir et al., 2017; Zeller et al., 2006) (Table S3).

However, the prevalence of individual pathogenic variants in an HCM population appears to be very low (Marian & Braunwald, 2017). The majority of other pathogenic variants occur at a frequency of <0.01 in the HCM population and nearly half are detected in a single proband or family(Alfares et al., 2015). Our data support these findings in our population, as most of the pathogenic variants identified here were detected in single probands. We found only one pathogenic variant, rs397516037 in *MYBPC3*, in two probands, which represented 2% of all non-related cases of HCM in our sample. This variant is rare in general population. However, Tóth T. et al. reported results that were similar to ours (Toth et al., 2011). The high occurrence of this pathogenic variant in our sample and various other cohorts (Ehlermann et al., 2008; Erdmann et al., 2001, 2003; Fokstuen et al., 2008; Ingles et al., 2005; Kapplinger et al., 2014; Toth et al., 2011; Zeller et al., 2006) might reflect "hot spots" for pathogenic variants or probably a founder effect (Page et al., 2012), which was confirmed by Erdmann et al. (Erdmann et al., 2001).

The other pathogenic variant in *MYBPC3* which leads to formation of the stop codon p.Y847X is also very rare even in individuals with HCM. This variant was also identified in patients from diverse populations (Berge & Leren, 2014; Chan et al., 2014; Kapplinger et al., 2014; Marsiglia et al., 2013; Zhao et al., 2017). Thus, this pathogenic variant in *MYBPC3* is responsible for the development of less than 1% of all cases of this condition in general population. The higher occurrence of this pathogenic variant in our population can be explained either by the peculiarities of the Russian population or by the small size of our sample.

The same is true for rs200411226 which leads to p.R495Q substitution in the MYBPC3 protein. Its frequency is also very low even in individuals with HCM. This pathogenic variant occurs on average in one patient out of 100, ranging from 0.5% to 8% (Brito et al., 2012; Christiaans et al., 2010; Ehlermann et al., 2008; Fokstuen et al., 2008, 2011; Helms et al., 2014; Kapplinger et al., 2014; Lopes et al., 2013; Maron et al., 2001; Marsiglia et al., 2013; Millat et al., 2010; Ng et al., 2013; Niimura et al., 1998; Van Driest et al., 2004; Zeller et al., 2006). Our data indicate that our population does not differ much from the European population in the case of this variant.

The pathogenic variant in the *MYH7* gene, which leads to the p.G741R substitution, was first associated with the development of HCM in Chinese patients by Song et al (Song et al., 2005). This pathogenic variant was also discovered in less than 0.01 in other samples worldwide(Berge & Leren, 2014; Garcia-Giustiniani et al., 2015; Kapplinger et al., 2014; Kaski et al., 2009; Marsiglia et al., 2013; Miller et al., 2013; Murphy et al., 2016; Otsuka et al., 2012; Perrot et al., 2005), whereas it was 1 out of 98 in patients with HCM in our sample.

As it was mentioned earlier also, we identified several probably damaging variants in *MYH7*, *MYBPC3*, *MYL3*, and *TPM1* genes, which are considered to be definitive HCM genes, as pathogenic variants in these genes are responsible for the development of HCM in the majority of cases of this condition (Table 3). These variants are also very rare, as they occur exclusively in single cases worldwide. Therefore, it is very difficult to prove their pathogenic significance by co-segregation analysis. However, some variants in the *MYBPC3* gene were classified as likely pathogenic.

As mentioned above, we also detected 13 probably damaging (that could affect a protein structure) variants in the moderate (*ACTN2*) and limited (*LDB3*, *TTN*, *MYH6*, *NEXN*, and *VCL*) genes of HCM, which may be causative pathogenic variants of HCM in 24 patients from our sample (Table 4). Computational prediction tools and a conservation analysis suggested that these variants have an impact on the structures of the proteins, although this information is not sufficiently predictive to determine pathogenicity, i.e., the clinical significance of these variants is uncertain (Ng et al., 2013). Some of these genes encode proteins that are structural components of sarcomere (*ACTN2*, *TTN*, *MYH6*), whereas others are linked to the sarcomere structurally (*NEXN*) or are involved in the maintenance of sarcomere function (*LDB3*, *VCL*). Several of the variants were detected in the same patients (Table S2). This fact may complicate the definition of pathogenicity of these variants. In general, there are few data on these extremely rare variants, which we identified, and much less on their involvement in the pathogenesis of HCM (Chen et al., 2012; Ploski et al., 2014; Walsh, Thomson, et al., 2017). Moreover, the frequencies of some of these variants were significantly higher in our sample than they were in the general population (Table 4). This fact may indicate both the potential pathogenicity of these variants and their ethno-specificity for our population. It is possible that these variants do not lead to the development of HCM by themselves; rather, their combination with another similar variant may participate in the pathogenic process.

In summary, further investigation of these potentially pathogenic variants is needed to prove their causal role in the pathogenesis of HCM.

5 | CONCLUSIONS

In this study, we described partially the spectrum of pathogenic variants that cause HCM in the Russian population.

Most of the pathogenic variants in our patients were detected in the *MYBPC3* and *MYH7* genes. The total frequency of pathogenic and likely pathogenic variants in our sample was 8%. However, the overall prevalence of individual pathogenic variants identified in our population appeared to be very low. Nevertheless, we were able to detect some novel likely pathogenic variants in HCM genes in our sample. We also identified 20 variants of uncertain significance in all HCM-related genes: 7 variants in the definitive genes and 13 variants in the moderate and limited genes.

It should be mentioned that, despite the fact that the exact pathogenic variants identified are not unique to our sample and are found in patients worldwide, the frequencies of these variants in our population differ slightly from those reported in Europe. This could be explained by ethno-specific traits of our population. Moreover, some rare probably damaging variants, especially those that were detected for the first time, could also be specific to our population. In addition, our findings may be explained by the features of our sample (mainly middle-aged patients without family history of the disease) and unidentified pathogenic variants in genes previously not linked with HCM, which alone or in combination could result in manifestation of mild form of HCM.

Since the initial discovery of the hereditary nature of HCM in the late 60s, many HCM causative genes have been discovered. However, it should be emphasized that, for a fairly large number of rare pathogenic variants, and despite the demonstrated connection with the development of HCM, there is still no reliable confirmation of a direct causal relationship between the variant and the disease. Furthermore, despite many years of research, genes that may be associated with the development of this disease remain undiscovered; the genetic basis of HCM remains undetected in at least one quarter of all cases of this condition[82]. Therefore, it is necessary to continue the search for new genes associated with the development of HCM using modern methods of genome-wide analysis, as well as molecular genetics and cellular technologies.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SPA and SMI developed the concept of the study. FEV, KNS, PNG, SPA, and SMI organized and coordinated the study. FEV, KNS, VIN, MMS, SPA, and SMI conducted the study and analyzed the data. FEV wrote the manuscript. SPA and SMI reviewed and revised the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethics Committees of RNRMU and Institute of Molecular Genetics. Written informed consent was obtained from all participating patients and families according to the Declaration of Helsinki.

PATIENT CONSENT FOR PUBLICATION

Written informed consent for publication was obtained from all participating patients and families.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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