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THE P.ARG230HIS VARIANT OF THE VCL PROTEIN IS NOT PATHOGENIC AND DOES NOT AFFECT HYPERTROPHIC CARDIOMYOPATHY PHENOTYPE IN RUSSIAN FAMILY CARRYING THE P.GLN1233TER PATHOGENIC VARIANT IN THE MYBPC3 GENE

Filatova Elena V., PhD*[1], Krylova Natalia S., PhD*[2], Kovalevskaya Elena A.[3], Maslova Maria Yu.[2], Poteshkina Natalia G. , PhD[2], Slominsky Petr A., Proff.[1], Shadrina Maria I., PhD[1]

[1]Institute of Molecular Genetics of National Research Centre «Kurchatov Institute», 123182, 2 Kurchatov Sq., Moscow, Russia

[2]Pirogov Russian National Research Medical University, 117997, 1 Ostrovitianov str., Moscow, Russia

[3]City Hospital № 52, 123182, 3 Pekhotnaya str., Moscow, Russia

* - equal contribution.

Corresponding author:
Filatova Elena V.,
Address: 2 Kurchatov Sq., Moscow, 123182, Russia,
e-mail: FilatovaEV@img.msk.ru,
phone: +7 499 1960224, fax: +7 499 1960221

Abstract

Background. Despite many years of research, the genetic basis of hypertrophic cardiomyopathy (HCM) remains poorly understood. The rapidly accumulating data from next-generation sequencing have not provided answers to all the questions that have arisen. Other research methods are needed to prove the involvement of the variants identified in pathogenesis of HCM.

Aims. We aimed to identify any specific clinical characteristics caused by a combination of two variants, rs397516037 in the MYBPC3 gene and rs749628307 in the VCL gene, in a Russian family of carriers and attempted to clarify whether the variant in the VCL gene contributes significantly to the development of HCM.

Methods. The studied family included three patients with HCM and one healthy family member. A targeted exome analysis was performed for the daughter of the proband. A structural alignment of both forms of VCL, the canonical form and the form with p.Arg230His substitution, was performed.

Results. The pathogenic variant, rs397516037, was detected in the proband and in several family members. A possibly damaging variant rs749628307 was detected in the proband and several family members investigated in this study. The structural alignment suggests that the rs749628307 variant does not alter the protein structure significantly and may not lead to impairment or loss of function.

Conclusions. Our analysis showed that, apparently, the rs749628307 variant in the VCL gene does not change the structure of the protein significantly or affect the severity and form of the clinical manifestations of HCM; therefore, it could not be considered as being pathogenic.

Keywords: Clinical characteristics, Genetics, Hypertrophic cardiomyopathy, Myosin binding protein C3, Pathogenic variant, Vinculin

Introduction

Hypertrophic cardiomyopathy (HCM) is classically characterized by the presence of idiopathic left ventricular hypertrophy and is probably the most common heritable cardiovascular disease with an estimated prevalence of up to 1:200 worldwide.[1] Clinically, HCM is very heterogeneous "and manifests in the form of varying degrees of hypertrophy, fibrosis, myocyte disarray, left ventricular outflow tract obstruction, ventricular septal morphology, associated symptoms, sudden cardiac death (SCD) susceptibility",[2] and "heart failure and embolic stroke secondary to atrial fibrillation".[3] It is predominantly transmitted in an autosomal dominant manner[3-6] with "sporadic cases associated with de novo mutations"[6, 7] and some case exhibiting maternally inheritance HCM.[8-11]

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Currently, more than 30 different HCM types are described in the Online Mendelian Inheritance in Man (OMIM) database (http://omim.org/phenotypicSeries/PS192600, last access 24.08.2021); these types are associated with 27 different mutant genes, mostly encoding various proteins of the sarcomere.

The myosin-binding protein C gene (*MYBPC3*) is one of the most frequently involved genes in patients with HCM, as mutations affecting it can be found in 15%–25% of patients with HCM.[12-15] A pathogenic variant, rs397516037, is responsible for the formation of a nonsense mutation and the synthesis of a truncated protein (p.Gln1233Ter). The incidence of this mutation in patients with HCM varies from <1% to almost 7% in different populations;[13, 16-22] therefore, it could be considered a relatively frequent disease-causing mutation.

Several pathogenic variants in the vinculin (*VCL*) gene have also been associated with the development of dilated and hypertrophic cardiomyopathies (https://omim.org/entry/193065, last access 24.08.2021). However, these are single cases worldwide that are not supported by co-segregation analyses or studies in cell or animal models. Therefore, it is difficult to describe a mechanism of pathogenesis of HCM or relevant clinical features associated with mutations in the VCL gene.

Based on the reports mentioned above we aimed to identify any specific clinical characteristics that are caused by a combination of two variants, rs397516037 in the *MYBPC3* gene and rs749628307 in the *VCL* gene, in a Russian family.

Materials and methods

Patients

The studied family included three patients with HCM and one healthy family member. These individuals were Russians (of Slavic origin) from the Moscow region. The patients were selected and investigated according to the European diagnostic criteria for familial HCM (i.e., interventricular septal thickness (IVS) of 15 mm or more in the absence of other known causes of hypertrophy) by Krylova N.S., associate Professor of General Therapy at Pirogov Russian National Research Medical University (RNRMU), Cardiological Department of City Clinical Hospital No. 52 of the Moscow City Health Department. Written informed consent was obtained from all participating patients and family members according to the Declaration of Helsinki. This study was approved by the Ethics Committees of Pirogov Russian National Research Medical University and Institute of Molecular Genetics.

DNA preparation and sequencing

Genomic DNA was obtained from leukocytes using the Quick-DNA Miniprep Kit (Zymo Research, Irvine, USA), as recommended by the manufacturer. The concentration of isolated nucleic acids was measured using a Qubit fluorometer (Invitrogen, Thermo Fisher Scientific, USA) and a Quant-iT DNA BR Assay Kit, as recommended by the manufacturer. Sanger sequencing was performed by the Evrogen company (Moscow, Russia).

Analysis of the three-dimensional structure of the VCL protein

A prediction of the three-dimensional (3D) structure of the canonical form of VCL and the variant of this protein carrying the p.Arg230His substitution was performed using algorithms for the prediction of protein structure and function that are based on I-TASSER.[23-25]

A comparison of the predicted 3D structures of the canonical forms of VCL, both predicted and the crystal structure downloaded from the Protein Data Bank in Europe (PDBe), entry 6FUY (https://www.ebi.ac.uk/pdbe/entry/pdb/6FUY), with that of the variant of this protein with the p.Arg230His substitution was carried out using a structural alignment software, PyMOL Molecular Graphics System, Version 2.4.0 (Schrödinger, LLC).

Results

The family consisting of a 46 year-old woman (proband/index patient), her 63 year-old, mother, her daughter 24 year-old, and her 25 year-old son was recruited into the study.

The proband's mother suffered from dizziness, chest pain, palpitation, and breathlessness during exercise. HCM was first detected on echocardiogram (EchoCG) at the age of 41 years. The proband's grandfather probably had HCM and died suddenly. The proband's mother had a history of arterial hypertension (AH) for at least 15 years (Table 1). Her height was 1.62 m, her weight was 65 kg, and her body surface area (BSA) was 1.99 m2. Physical examination revealed normal lung auscultation data with a respiratory rate of 18 per min, a regular heart rate (HR) of 62 bpm, and a blood pressure (BP) of 160/80 mm Hg. The patient exhibited no signs of congestive heart failure (HF), but her NT-proBNP level was 2355 ng/l. The results of ECG and 24-h ECG monitoring (Table 2) revealed sinus rhythm with a horizontal position of the electrical axis of the heart and slight ST depression in I, aVL, and V6, with a negative T-wave in V4-V6. EchoCG revealed severe hypertrophy with maximal wall thickness at the basal and middle anterior septa (Table 3). Thus, the patient had the classic form of asymmetric nonobstructive HCM with severe left ventricle (LV) diastolic dysfunction (Table 1). The estimated risk of SCD was intermediate

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(4.5%); thus ICD was considered. Drug treatment included amiodarone (200 mg/day) for arrhythmias, and valsartan (160 mg/day) with indapamide (2.5 mg/day) and amlodipine (5 mg/day) for AH.

The proband was first diagnosed with HCM after EchoCG at the age of 38. EchoCG was performed to investigate the origin of chest pain, palpitation and breathlessness during exercise. The patient had no history of AH (Table 1). Her height was 1.64 m, her weight was 61 kg, and her BSA was 1.67 m^2. Physical examination revealed normal lung auscultation data with a respiratory rate of 18 per min, a systolic murmur along the left sternum side, with regular HR of 80 bpm and BP of 100/60 mm Hg. The patient had no signs of congestive HF, but her NT-proBNP level was 2921 ng/l. The results of ECG and 24-h ECG monitoring (Table 2) revealed sinus rhythm with a normal electrical axis of the heart and LV hypertrophy (Cornell criterion 34 mm). EchoCG also revealed the presence of severe hypertrophy (Figure 1). The proband had the classic form of asymmetric obstructive HCM with severe LV diastolic dysfunction (Table 3). An exercise ergometer test showed moderate exercise tolerance with normal blood pressure response without signs of ischemia. Stress-echoCG revealed an increase of LVOT obstruction, pulmonary hypertension, and diastolic dysfunction during exercise (Table 4). These changes together with a high LV mass may be responsible for the high level of NT-proBNP. The estimated risk of SCD was low (3.4%) and the patient did not need ICD, but cardiac surgery for LVOT gradient reduction was consider. Drug treatment was initiated with a beta-blocker (bisoprolol, 5 mg/day); however, because of hypotension (average BP, 100/58 mmHg with episodes of decrease of the BP to 80/46 mm Hg), the bisoprolol dose was reduced to 1.25 mg/day and ivabradin was started from 10 to 14 mg/day.

The son of the proband was 25 years old, with a height of 1.90 m, and weight of 68 kg. He exhibited no history of AH (Table 1), had one syncope episode in childhood, and rare dizziness. HCM was first diagnosed on EchoCG performed when he was 23 years of age during the investigation of his mother. He does not currently experience any symptoms of HCM during ordinary activity, and was placed in the New York Heart Association Functional Class 1 regarding the degree of heart failure. ECG showed sinus rhythm with a normal electrical axis of the heart and was quite normal (Table 2). Moreover, 24-h ECG monitoring appeared to show no deviations (Table 2). An exercise ergometer test showed that the patient had high exercise tolerance with normal BP response, but a prolonged recovery period because of HR restoration (Table 4). However, EchoCG revealed severe hypertrophy (Table 3). The estimated risk of SCD was low (3.3%), and the patient does not currently need ICD, cardiac surgery, or any drug therapy.

The daughter of the proband did not experience any symptoms of HCM, both at rest and during exercise, at the time of enrollment, and her ECG appeared to be normal; therefore, she did not undergo 24-h ECG monitoring, EchoCG, or the exercise ergometer test.

A targeted exome analysis was performed in the proband to determine the genetic cause of the disorder. The genetic analysis revealed a disease-causing pathogenic variant, rs397516037, according to the ClinGen resource(26), leading to the replacement of a glutamine residue (Gln) with a stop codon (Ter) (p.Gln1233Ter) in the *MYBPC3* gene in the proband and in several family members (Figure 2).

A possibly damaging variant rs749628307, leading to the p.Arg230His amino acid substitution was also detected in the *VCL* gene in the proband; therefore, it was verified by Sanger sequencing in all family members investigated in this study (Figure 2). A bioinformatics analysis showed that this variant could be damaging according to SIFT score (0), PolyPhen-2 score (0.999), REVEL score (0.795), and CADD phred (32). Therefore, an analysis of the 3D structure of the VCL protein, both of the canonical form and the form with the p.Arg230His substitution, was performed using the I-TASSER online platform. The structural alignment of both forms of VCL, i.e., the canonical form and that with the p.Arg230His substitution (Figure 3) suggests that the rs749628307 variant does not alter the protein structure significantly and may not lead to impairment or loss of function of the protein.

Discussion

The HCM-causing mutation in the family studied here was p.Gln1233Ter (rs397516037 in the MYBPC3 gene). There are multiple lines of evidence of the pathogenicity of this variant.[13, 17, 18, 21, 22] It is currently considered pathogenic according to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/42735/, last access 24.01.2021) and the American College of Medical Genetics and Genomics guidelines.[27]

However, to the best of our knowledge, no report has described any correlation between the clinical manifestations of HCM and this mutation, probably because few patients carrying this variant have been investigated, and the data on the subject are scarce. Nevertheless, it seems that the phenotype caused by this mutation may be modulated by gender (Table 5). Despite the fact that male and female patients are equally affected, the mean IVS was 23.5 in females and 18.5 in males. It is worth noting that IVS most likely does not depend on age of onset: 21.5 in younger (45 years or less), and 21.6 in older (46 years or older) patients. In general, carriers of this mutation have a mild course of the disease and a low risk of

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sudden cardiac death; however, the implantation of a cardioverter-defibrillator or a resection of IVS could be recommended in some cases.

We also suggested that the presence of another possibly damaging variant could affect the clinical phenotype caused by p.Gln1233Ter (rs397516037). The detection of the possibly damaging variant rs749628307 in the *VCL* gene in the proband of our study raised the possibility that this variant could affect the clinical picture of the disease. Unfortunately, the data on rs749628307 in dbSNP and ClinVar databases are limited. To our knowledge, it has not been described in any publication. This is a very rare variant, with a MAF of 0.000012 according to the GnomAD_exome database.

Therefore, we attempted to evaluate the pathogenetic significance of this variant. First, we analyzed the presence of rs749628307 in all available members of the family studied here. The possibly damaging allele of this variant did not co-segregate with the disease. The fact that the daughter of the proband was the sole healthy carrier of this variant could be explained in several ways: 1) this variant is not pathogenic; 2) the changes in the VCL protein structure are very subtle and cannot cause the development of HCM alone; 3) the daughter of the proband is young and the disease did not yet manifest itself; and 4) the variant has incomplete penetrance.

Thus, we decided to check whether the p.Arg230His amino acid substitution affects the 3D structure of the protein. To test this hypothesis, we performed a structural alignment analysis of the 3D structures of conservative and mutated forms of the VCL protein (Figures 3A and 3B). We found that the p.Arg230His amino acid substitution does not affect the 3D structure of the VCL protein, at least according to the predictions.

Thus, the presence of this variant in the healthy family member (the daughter of the proband) and the analysis of the 3D structure demonstrating that p.Arg230His does not alter the structure of the VCL protein suggest that the rs749628307 variant is benign.

Conclusion

Despite many years of research, the genetic basis of HCM remains poorly understood. However, even the rapidly accumulating data from next-generation sequencing and related computer analyses to predict the effect of the identified variants on the protein structure have are not provided answers to all the questions that have arisen. Therefore, segregation analysis and analysis of the 3D structure of the whole protein are still relevant to prove or to rule out the pathogenicity of the identified variants.

In this study, we investigated whether the rs749628307 variant in the VCL gene, which leads to the p.Arg230His amino acid substitution, affects the structure of the protein and the clinical features of patients with HCM, carrying the disease-causing variant rs397516037 in the *MYBPC3* gene. Our analysis showed that, apparently, the rs749628307 variant in the *VCL* gene does not change the structure of the protein significantly or affect the severity and form of the clinical manifestations of HCM.

List of abbreviations

HCM – hypertrophic cardiomyopathy; VCL – vinculin; MAF – minor allele frequency; MYBPC3 – myosin-binding protein C; EchoCG – echocardiography; MVA –mitral valve annulus; LV – left ventricle; RV – right ventricle; LVOT – Left ventricular outflow tract. IVS – interventricular septum; AV Vmax – aortic valve maximum velocity; AV Vmean – aortic valve mean velocity; AV maxPG – aortic valve maximum pressure gradient; AV meaPG – aortic valve mean pressure gradient; AV VTI – aortic velocity time integral; AV Env.Ti – aortic velocity envelope time; HR – heart rate; BPM – beats per minute; ICD – implantable cardioverter defibrillator; OMIM – Online Mendelian Inheritance in Man; BSA – body surface area; HR – heart rate; BP – blood pressure; HF – heart failure; ECG – electrocardiography; SCD – sudden cardiac death; 3D – three-dimensional; AH – arterial hypertension; Gln – glutamine residue; Ter – stop codon; PDBe – Protein Data Bank in Europe; Arg – arginine residue; His – histidine residue.

Declarations

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.

Consent for publication

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

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Competing interests

The authors declare that there is no conflict of interest.

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Authors' contribution

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

Authors' information

FEV and KNS contributed equally to this work.

Figures and tables captions and legends

Figure 1. Echocardiography of the proband, 46 years old.

A - Parasternal position, long axis view of LV. **B** – Pulse wave doppler ecocardiography, LVOT obstruction with maximum gradient 76 mmHg. IVS – interventricular septum; AV Vmax — aortic valve maximum velocity; AV Vmean — aortic valve mean velocity; AV maxPG — aortic valve maximum pressure gradient; AV meaPG - aortic valve mean pressure gradient; AV VTI - aortic velocity time integral; AV Env.Ti — aortic velocity envelope time; HR — heart rate; BPM — beats per minute. (reproduction size: at full page width)

Figure 2. Pedigree of the family.

Squares and circles denote males or females, respectively; filled symbols represent clinically affected family members. Deceased individuals are slashed. Symbol painted in stripes represents a family member possibly clinically affected. (reproduction size: at column width)

Figure 3. The comparison of 3D structures of the forms of VCL.

A - The comparison of 3D structures of canonical form of VCL (crystal structure downloaded from Protein Data Bank in Europe (PDBe), entry 6FUY) and the variant of this protein with p.Arg230His substitution. **B** - The comparison of predicted 3D structures of canonical form of the VCL protein and the variant of this protein with the p.Arg230His substitution. The 3D structure of canonical form of the VCL protein – blue; The 3D structure of the variant of this protein with the p.Arg230His substitution – magenta; His – green; Arg – red. (reproduction size: at full page width)

Table 1. 24-hours BP-monitoring parameters of 3 members of the family with HCM

Table 2. 24-hours ECG-monitoring parameters of 3 members of the family with HCM

Table 3. EchoCG parameters of 3 members of the family with HCM

EchoCG – echocardiography, MVA-mitral valve annulus, LV – left ventricle, RV – right ventricle

Table 4. Exercise test parameters of the proband and her son with HCM

Table 5. The main HCM diagnostic criterion in patients with p.Gln1233Ter mutation form different populations.

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