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No Effect of the p.Arg230His Variant Of The VCL Protein on the Course of the Hypertrophic Cardiomyopathy In Russian Family Carrying The p.Gln1233Ter Pathogenic Variant In The MYBPC3 Gene

Aim	To determine specific clinical characteristics caused by a combination of the rs397516037 pathogenic variant in the myosin-binding protein C (MTBPC3) and the rs749628307 polymorphic variant in the vinculin (VCL) gene in a Russian family of carriers and to evaluate the contribution of the rs749628307 polymorphic variant in the VCL gene to the development of hypertrophic cardiomyopathy (HCMP).
Material and Methods	The family under study included one healthy person and 3 patients with HCMP. A targeted analysis of proband's exome was performed. A structural alignment for both forms of the VCL protein, the canonical form and the form with p.Arg230His substitution, was performed.
Results	The pathogenic rs397516037 variant and the potentially pathogenic rs749628307 variant were detected in the proband and several family members. A possibly damaging variant rs749628307 was detected in the proband and several family members evaluated in this study. The structural alignment confirmed that the rs749628307 variant did not alter the protein structure significantly and could not cause an impairment or loss of the protein function.
Conclusion	This study demonstrated that apparently the rs749628307 variant in the VCL gene does not affect the protein structure in a pathogenetically significant way, neither does it affect the severity and form of the clinical manifestations of HCMP; therefore, it cannot be considered as pathogenic.

Keywords	Clinical characteristics; genetics; hypertrophic cardiomyopathy; myosin-binding protein C; pathogenic variants; vinculin
For citations	Filatova E.V., Krylova N.S., Klass A.L., Kovalevskaya E.A., Maslova M.Yu., Shadrina M.I. et al. No Effect of the p.Arg230His Variant Of The VCL Protein on the Course of the Hypertrophic Cardiomyopathy In Russian Family Carrying The p.Gln1233Ter Pathogenic Variant In The MYBPC3 Gene. Kardiologiia. 2023;63(3):28–35. [Russian: Филатова Е.В., Крылова Н.С., Класс А.Л., Ковалевская Е.А., Маслова М.Ю., Шадрина М.И. и др. Отсутствие влияния варианта p.Arg230His (rs749628307) в гене VCL на течение гипертрофической кардиомиопатии в русской семье носителей патогенного варианта p.Gln1233Ter (rs397516037) в гене MYBPC3. Кардиология. 2023;63(3):28–35].

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Hypertrophic cardiomyopathy (HCM) is traditionally characterized by the presence of left ventricular (LV) myocardial hypertrophy. The estimated global prevalence of the disease is 1 in 200, which makes HCM probably the most common hereditary cardiovascular disease [1]. The morphological picture of HCM is very heterogeneous and manifests as myocardial hypertrophy of any degree, most often of the interventricular septum (IVS), disordered arrangement of cardiomyocytes, fibrosis, and the possible presence of intraventricular obstruction. The disease can clinically manifest as dyspnea, fainting, angina, reduced exercise tolerance, and an increased risk of sudden cardiac death (SCD) due to ventricular arrhythmias. The development of atrial fibrillation with clinically evident heart failure (HF) and embolic complications is a more typical scenario for elderly patients with HCM [2, 3]. HCM is mainly associated with autosomal dominant inheritance [3–6], sporadic cases caused by de novo mutations [6, 7], and cases of maternal inheritance [8–11].

Currently, the Online Mendelian Inheritance in Man (OMIM) compendium contains more than 30 different genetic variants of HCM (http://omim.org/phenotypicSeries/PS192600, last accessed 20.04.2022); these types are associated with 27 mutant genes that mainly encode sarcomere proteins.

The cardiac myosin binding protein C (MYBPC3) gene is considered one of the most frequently involved in the development of HCM. Mutations of this gene are found in 15–25% of patients [12–15]. The pathogenic

ISSN 0022-9040. Kardiologiia. 2023;63(3). DOI: 10.18087/cardio.2023.3.n1937

variant, rs397516037, is a nonsense mutation that results in the synthesis of a shorter form of the protein (p.Gln1233Ter). The prevalence of this mutation in patients with HCM ranges from 1% to 7% in different populations [13, 16–22]; thus, this variant can be considered a relatively common cause of the disease.

Some pathogenic variants in the vinculin gene (VCL) can also be associated with the development of dilated and hypertrophic cardiomyopathy (https://omim.org/entry/193065, last accessed 20/04/2022). However, the isolated cases worldwide are not confirmed by co-segregation analysis or studies in cell or animal models, which complicates the description of the pathogenic mechanism of HCM, and related clinical signs caused by VCL mutations.

In this regard, the objective of this study was to determine the specific clinical characteristics caused by the combination of rs397516037 in MYBPC3 and rs749628307 in VCL in a Russian family, and the role of the rs749628307 polymorphic variant in the VCL gene in the pathogenesis of HCM.

Material and Methods

The family under study included 3 patients with HCM and one healthy family member. All subjects were Russians (Slavic ethnic origin) from the Moscow region. Patients were selected and examined in accordance with the European diagnostic criteria for familial HCM (i.e., IVS thickness ≥15 mm in the absence of other causes of hypertrophy) in the cardiology department of the City Clinical Hospital No. 52 (Moscow, Russian Federation). Following the Declaration of Helsinki, all patients and their family members signed the informed consent. The study was approved by the Ethics Committee of the N. I. Pirogov Russian National Research Medical University (Minutes No.139 dated November 10, 2014).

Phenocopies of HCM were excluded in all patients [23].

DNA preparation and sequencing

Genomic DNA was isolated from peripheral blood leukocytes using the Quick-DNA Miniprep Kit following the manufacturer's recommendations. The concentrations of nucleic acid were measured using the Quant iT DNA BR Assay Kit and the Qubit fluorometer following the manufacturer's recommendations. Sanger sequencing was performed in the Eurogen laboratory.

Analysis of the three-dimensional structure of the VCL protein

Prediction of three-dimensional (3D) structures of the canonical form of VCL and the variant of the p.Arg230His substitution carrying protein was performed using the prediction algorithms for the protein structure and functions based on I-TASSER [24–26].

The 3D structures of canonical forms of VCL, predicted and crystal forms, downloaded from the European Protein Data Bank (PDBe), entry 6FUY (https://www.ebi.ac.uk/pdbe/entry/pdb/6FUY), were compared with the predicted variant of the protein with amino acid substitution p.Arg230His using structural alignment software (PyMOL Molecular Graphic System v. 2.4.0 (Schrödinger, LLC)).

Results

The study included a family consisting of a 46 year-old woman (proband/index patient), her 67 year-old mother, her 24 year-old daughter, and 25 year-old son.

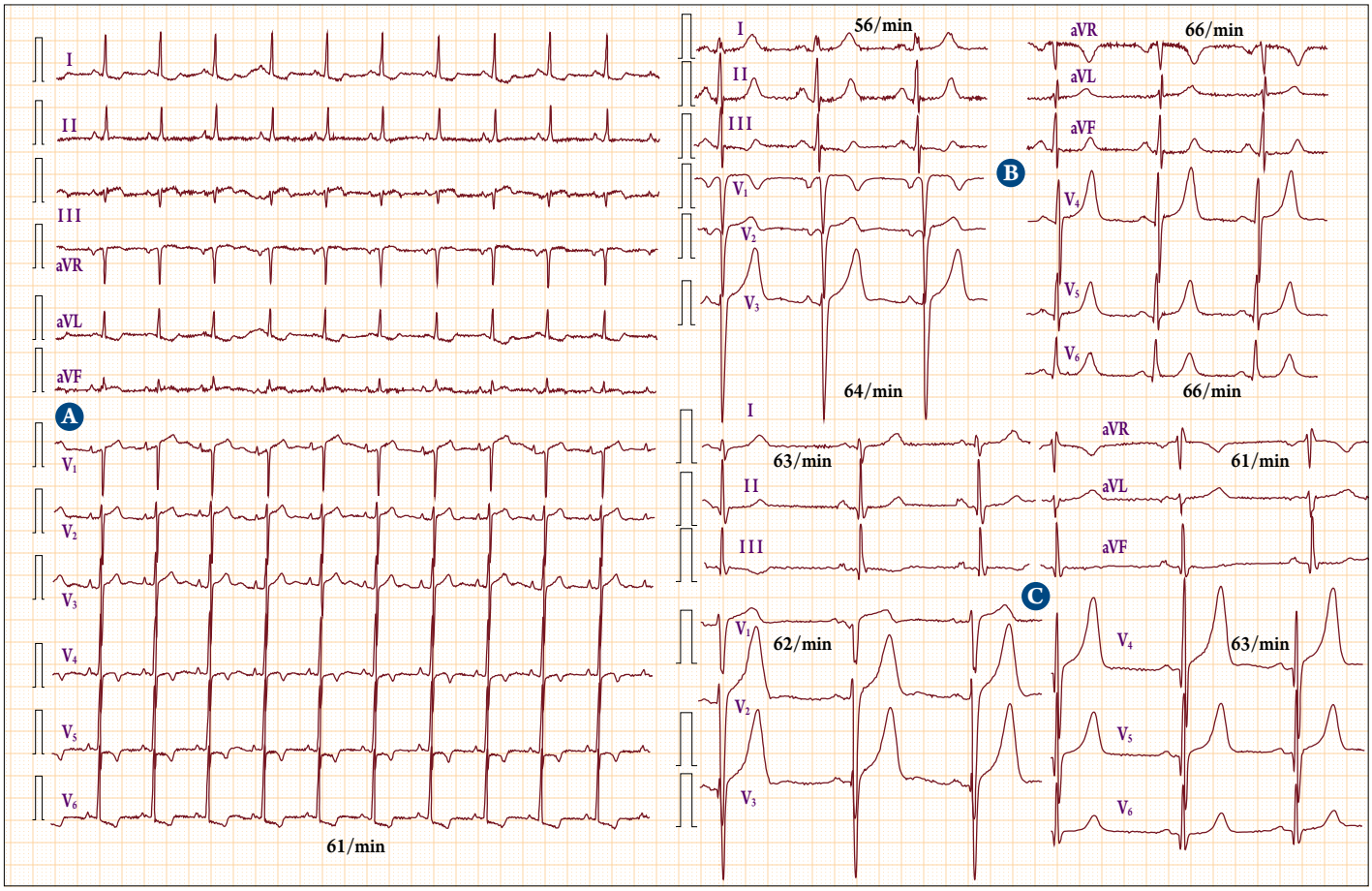
The mother of the proband complained of dizziness, non-exercise-related chest pain, palpitations, and dyspnea during exercise. It is known from the family history that the father of the proband's mother died suddenly. HCM was first detected during echocardiography when the patient was 41 years old. The woman had a 15-year-long history of arterial hypertension (AH) (Table 1). According to the results of the physical examination, the height was

Table 1. Results of 24-hour BP monitoring of three members of a family with HCM

Parameter	Mother of the proband, 67 y.o.	Proband, 46 y.o.	Son of the proband, 25 y.o.
BP, mm Hg			
• Mean daytime SBP	156	116	110
• Mean daytime DBP	60	65	61
• Mean nighttime SBP	152	100	95
• Mean nighttime DBP	58	56	50
• Maximum SBP	213	143	137
• Maximum DBP	106	82	75
Nighttime reduction in SBP, %	3	14	13
Nighttime reduction in DBP, %	3	15	18
Pulse wave velocity in the aorta, m/s	12.3	9.5	14.3
Mean pulse pressure, mm Hg	96	49	48

HCM, hypertrophic cardiomyopathy.

Figure 1. ECG of patients with hypertrophic cardiomyopathy



A – mother of the proband, 67 y.o.; **B** – proband, 46 y.o.; **C** – son of the proband, 25 y.o. HCM, hypertrophic cardiomyopathy.

Table 2. Results of 24-hour ECG monitoring of three members of the family with HCM

Parameter	Mother of the proband, 67 y.o.	Proband, 46 y.o.	Son of the proband, 25 y.o.
HR, bpm			
• Maximum	80	131	148
• Minimum	52	45	41
• Mean	62	72	70
Total number of supraventricular extrasystoles	539	14	0
Total number of ventricular extrasystoles	2,801	21	0
Episodes of supraventricular tachycardia	4	0	0
Episodes of ventricular tachycardia	3	0	0
ST-segment depression	No	No	No

HCM, hypertrophic cardiomyopathy.

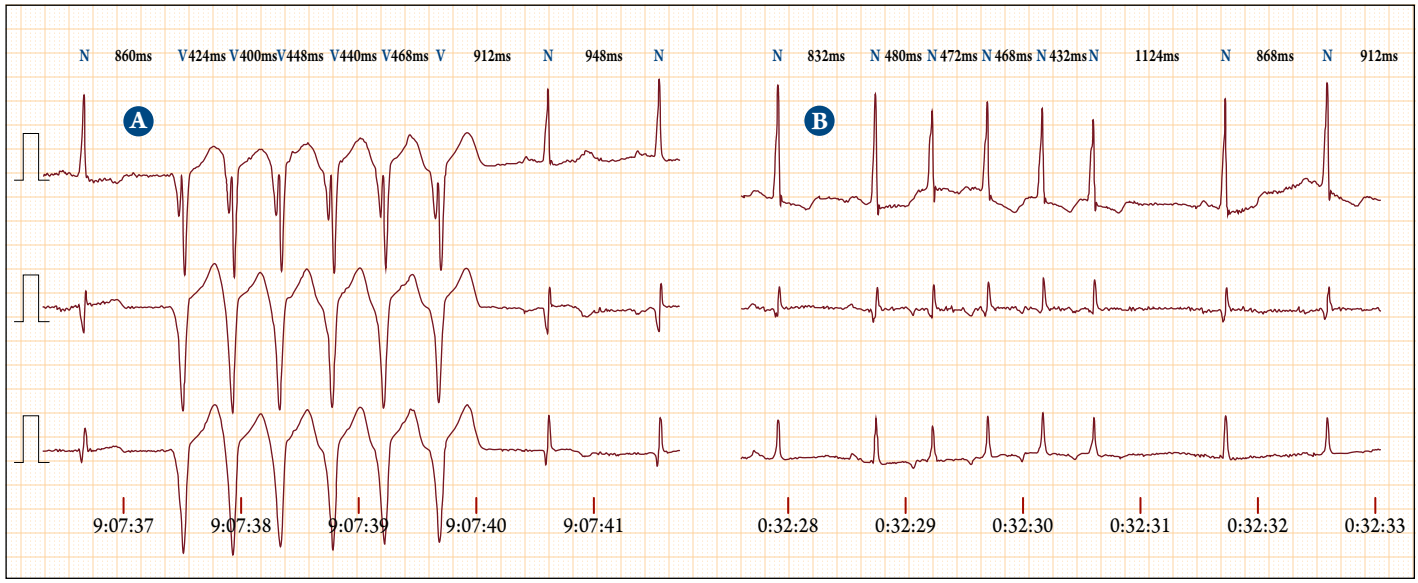
1.62 m, body weight was 65 kg, and body surface area (BSA) was 1.99 m^2. Auscultation showed vesicular breathing, no rale, and respiratory rate (RR) of 18 breaths per minute. Heart sounds were clear, heart rhythm was regular, heart rate (HR) was 62 bpm, blood pressure (BP) is 160/80 mm Hg. The patient had no objective signs of congestive HF, but the level of N-terminal pro-brain natriuretic peptide (NT-proBNP) was as high as 2,355 ng/L. The electrocardiogram (ECG) showed the horizontal position of the electrical heart axis, sinus rhythm, a slight ST-segment depression in leads I, aVL, and V6, a negative T-wave in leads V4–V6 (Figure 1, A). The 24-hour ECG monitoring revealed many episodes of ventricular and supraventricular extrasystoles (Table 2), and several episodes of ventricular and supraventricular tachycardia (Figure 2).

Echocardiography showed severe LV hypertrophy with maximum thickening of the basal and mid IVS segments (Table 3), and moderate mitral regurgitation. Thus, the patient had a classic form of asymmetric non-obstructive HCM with severe LV diastolic dysfunction (Table 3).

The risk of SCD was intermediate (4.5%). The patient was referred to a cardiac surgeon to consult on installing an implantable cardioverter-defibrillator (ICD). Pharmacotherapy included amiodarone (200 mg/day) for arrhyth-

ISSN 0022-9040. Kardiologiia. 2023;63(3). DOI: 10.18087/cardio.2023.3.n1937

Figure 2. Fragment of 24-hour ECG monitoring of the mother of the proband, 67 y.o.



Episodes of ventricular (**A**) and supraventricular (**B**) tachycardia.

Table 3. Echocardiography of three members of the family with HCM

Parameter	Normal range	Mother of the proband, 67 y.o.	Proband, 46 y.o.	Son of the proband, 25 y.o.
Interventricular septum, mm	6–10 (M); 6–9 (F)	19	22	15
LV inferior wall, mm	6–10 (M); 6–9 (F)	12	12	9
LV end-diastolic volume, mL	62–150 (M); 46–106 (F)	95	69	73
LV end-systolic volume, mL	21–61 (M); 14–42 (F)	33	19	27
LV end-diastolic volume index, mL/m^2	34–74 (M); 29–61 (F)	48	42	38
LV end-systolic volume index, mL/m^2	11–31 (M); 8–24 (F)	17	11	14
LV stroke volume index, mL/m^2	> 35	31	30	24
LVEF, %	52–72 (M); 54–74 (F)	65	70	63
LA diameter, mm (parasternal view)	< 40	45	46	33
LA volume index, mL/m^2	16–34	38	35	29
Mean pulmonary artery pressure, mm Hg	9–14	14	11	9
E/A	0.8–2.0	3.0	2.5	3.0
E/e’	< 8	14	12	4.8
LV Tei index	–	0.50	0.42	0.51
RV Tei index	< 0.43	0.40	0.56	0.35
Septal MVA e’ – a’ – s’, m/s	e’>7; a’>10	6–4–5	7–6–8	16–10–11
Degree of LV systolic dysfunction	–	3	2	0
Maximum LV outflow pressure gradient, mm Hg	< 30	8	76	5

HCM, hypertrophic cardiomyopathy; LA, left atrium; LV, left ventricle.

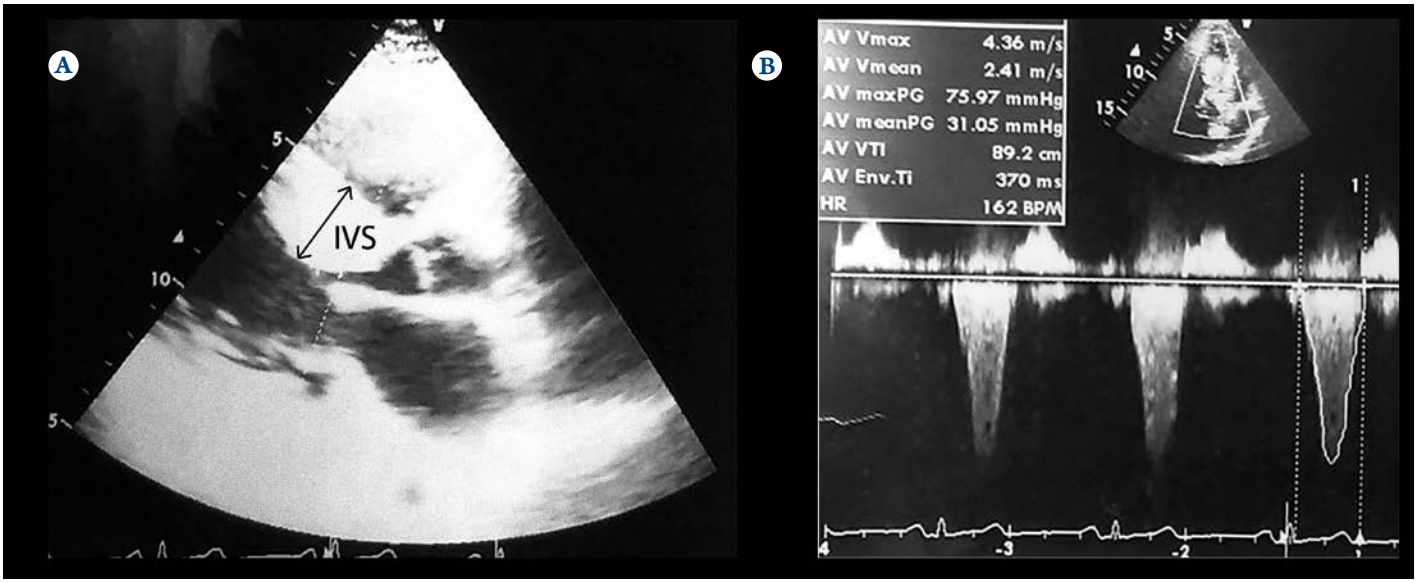
mia, and valsartan (160 mg/day), indapamide (2.5 mg/day), and amlodipine (5 mg/day) for AH.

HCM was newly diagnosed by echocardiography when the proband was 38 years old and complained of chest pain, palpitations, and dyspnea during exercise. No data confirming AH were obtained (Table 1). The patient’s height was 1.64 m, body weight was 61 kg, and BSA was 1.67 m^2. Vesicular breathing, no rale, RR 18 breaths per minute. Heart sounds were clear, heart rhythm was correct, HR was 80 bpm. Loud systolic murmur was heard on the left edge of the sternum. BP 100/60 mm Hg. The patient had no signs of congestive HF, but the level of NT-proBNP reached 2,921 ng/L.

ECG showed the horizontal position of the electrical heart axis, sinus rhythm, signs of LV hypertrophy (Cornell voltage index 34 mm; Figure 1, B).

Echocardiography showed severe asymmetric LV hypertrophy with thickening of the basal and mid IVS segments (Figure 3), and moderate mitral regurgitation. Thus, the proband had a classic form of asymmetric obstructive HCM with pronounced LV diastolic dysfunction (Table 3).

Figure 3. Echocardiogram, 46 y.o.



A – parasternal view along the long axis of the left ventricle;
B – continuous wave Doppler, maximum left ventricular outflow gradient is 76 mm Hg.

Ergometry exercise test showed average exercise tolerance with an adequate BP response without signs of myocardial ischemia (Table 4).

Stress echocardiography showed more severe obstruction in the LV outflow tract, pulmonary hypertension, and diastolic dysfunction during exercise, which may explain elevated NT-proBNP.

The estimated risk of SCD was low (3.4%), and the patient did not need ICD implantation, however, the surgery was suggested to reduce the LV outflow gradient. Drug treatment was initiated with beta-blockers (bisoprolol 5 mg/day), but due to hypotension (mean BP 100/58 mm Hg with episodes of a reduction to 80/46 mm Hg), the dose was reduced to 1.25 mg/day and ivabradine 10 mg/day was prescribed.

The son of the proband was 25 years old, his height was 1.9 m, and body weight was 68 kg. He had no history of AH (Table 1). One case of fainting in childhood and rare episodes of dizziness were reported. HCM was newly diagnosed by echocardiography at the age of 23 years, which was performed due to the diagnosis of HCM in his mother. At the time of the examination, the patient did not experience symptoms of HCM during normal activity, and HF of NYHA FC I was diagnosed. ECG (Figure 1, B) showed the normal position of the electrical heart axis, sinus rhythm, and no abnormalities.

Echocardiography revealed asymmetric LV hypertrophy with maximum thickening of the mid segments of the IVS. No intraventricular obstruction was detected. The heart cavities had normal dimensions. Systolic and diastolic functions of the LV were not impaired.

Parameters of 24-hour ECG monitoring remained normal (Table 2). Ergometry stress test showed that the patient had a high exercise tolerance and a normal BP

Table 4. Results of the ergometry exercise test of the proband and her son with HCM

Parameter	Proband, 46 y.o.	Son of the proband, 25 y.o.
HR at rest, bpm	66	87
HR at peak exercise, bpm	148	165
SBP/DBP at rest, mm Hg	100/60	100/60
SBP/DBP at peak exercise, mm Hg	140/70	140/50
Duration of exercise test, min	7.3	15
Reason for stopping the test	Submaximal HR, dyspnoea	Submaximal HR
BP recovery time, min	2	3
HR recovery time, min	10	9
Increase in LVSV during exercise, mL	15	-1
Increase in LVEF during exercise, %	5	1
Maximum LV outflow gradient at peak exercise, mm Hg	95	8

HCM, hypertrophic cardiomyopathy; LVSV, left ventricular stroke volume; LVEF, left ventricular ejection fraction; LV, left ventricle.

ISSN 0022-9040. Kardiologiia. 2023;63(3). DOI: 10.18087/cardio.2023.3.n1937

response, however, a recovery period was long due to longer HR normalization (Table 4).

The estimated risk of SCD was low (3.3%), and the patient did not need an ICD to be implanted, and there were no other indications for surgery or drug therapy.

The 24-year-old daughter of the proband had no symptoms of HCM at rest or during exercise, ECG and echocardiogram characteristics were normal, and 24-hour ECG monitoring and ergomentry stress test were not conducted.

We had previously performed a targeted exome analysis to identify the genetic cause of the proband's disease [23]. As a result, a pathogenic variant of rs397516037 was found, which leads to the replacement of the glutamine (Gln) residue with a stop codon (Ter) in the MYBPC3 gene according to ClinGen [27]. Genotyping of other family members with HCM also detected this pathogenic variant, rs397516037, leading to the development of HCM (Figure 4).

Moreover, the proband was found to have a potentially pathogenic variant, rs749628307, in the VCL gene, which leads to the amino acid substitution p.Arg230His. This variant was confirmed by Sanger sequencing to be present in all family members (Figure 4). The earlier bioinformatic analysis [23] showed that this variant can be damaging according to the following criteria: SIFT score (0), PolyPhen-2 score (0.999), REVEL score (0.795) and CADD phred (32) (additional Table 2 via reference [23]).

In this connection, the analysis of the 3D structure of the canonical form and the form with the p.Arg230His replacement of the VCL protein was carried out in I-TASSER. Structural alignment of both forms (Figure 5) confirmed that the rs749628307 variant does not significantly alter the structure of the protein and cannot lead to impairment or loss of function.

Discussion

The development of HCM in the family under study was caused by the rs397516037 mutation in MYBPC3, which leads to the amino acid substitution p.Gln1233Ter. There is a lot of evidence of the pathogenicity of this variant [13, 17, 18, 21, 22]. It is currently classified as pathogenic according to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/42735/, last accessed 20.04.2022) and the guidelines of the American College of Medical Genetics and Genomics [28].

However, there are no reports, to the best of our knowledge, which describe the relationship between the clinical features of HCM and this mutation, which may be due to the limited number of carriers examined and the lack of data. However, our analysis and the analysis of the world's literature suggest that the phenotype caused by this mutation may be sex related. Despite the fact that the pathology occurs in male and female patients with equal frequency, mean thickness of IVS in HCM is 23.5 mm in female patients and 18.5 mm in male patients. It should be noted that IVS thickness appears to not depend on the age of the disease manifestation: 21.5 mm in younger patients (45 years old and younger); 21.6 mm in patients of middle age and older (46 years old and older). Carriers of this mutation have generally a relatively mild course of the disease and a low risk of SCD. However, ICD or surgical resection of IVS is recommended in some cases.

We also suggest that the presence of other potentially pathogenic variants may affect the clinical phenotype caused by the p.Gln1233Ter replacement (rs397516037). In our study, the potentially pathogenic variant rs749628307 in the VCL gene identified in the proband is likely to affect the clinical picture of the disease. Unfortunately, there is insufficient information on rs749628307 in the dbSNP and ClinVar databases. As far as we know, cases of this variant have not yet been described. This variant is very rare, with a minor allele frequency (MAF) of 0.000012 according to the GnomAD_exome database.

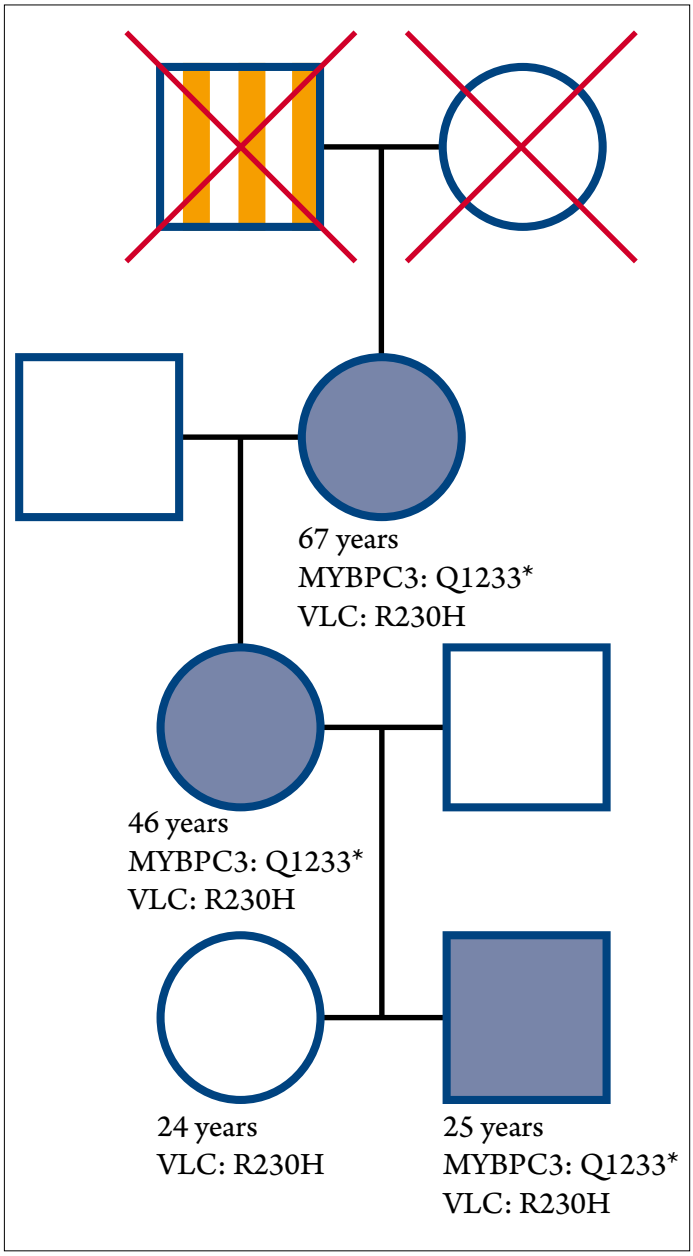
Thus, an attempt was made to assess the pathogenetic significance of this variant. The rs749628307 polymorphism was genotyped in all members of the family under study. As seen in Figure 4, the potentially pathogenic allele of this polymorphism does not co-segregate with the disease. There are several probable explanations of the fact that the daughter of the proband was the only healthy carrier of the variant:

1) this variant is not pathogenic;
2) changes in the VCL protein structure are very insignificant and cannot be an independent cause of HCM;
3) the daughter of the proband is young and the pathology has not yet manifested;
4) the variant has incomplete penetrance.

Therefore, it was decided to test whether the amino acid substitution p.Arg230His affects the 3D structure of the protein. The 3D structures of the conserved and mutant forms of the VCL protein were aligned to test this hypothesis (see Figure 5). It was found that the amino acid substitution p.Arg230His did not affect the 3D structure of the VCL protein, at least according to the predicted structures.

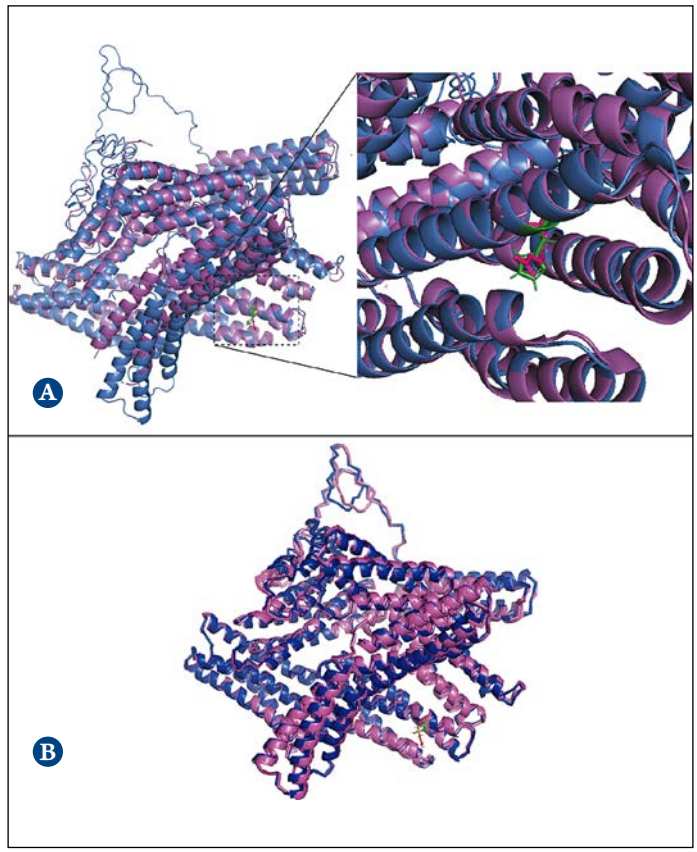
Thus, the benign (non-pathogenic) nature of the rs749628307 variant is confirmed by the presence of this variant in the healthy family member (the proband's daughter) and the 3D structure analysis demonstrating that the amino acid substitution p.Arg230His does not significantly change the VCL protein structure.

Figure 4. Pedigree of the family under study



Squares and circles denote men and women, respectively; solid symbols denote family members with clinically confirmed disease. Symbols representing the deceased family members are crossed out. Striped symbol denotes a family member who was likely to have the disease.

Figure 5. Comparison of 3D structures of the VCL protein forms



A – comparison of 3D structures of the VCL protein – the canonical form (crystal structure loaded from the European Protein Data Bank (PDBe), entry 6FUY) and predicted variant of the protein bearing amino acid substitution p.Arg230His; **B** – comparison of the predicted 3D structures of the VCL protein – the canonical form and the protein variant bearing amino acid substitution p.Arg230His. The 3D structure of the canonical form of VCL is blue; the 3D structure of the amino acid substitution variant of the protein is purple; the amino acid residue of histidine (His) is green; arginine (Arg) is red.

Conclusion

Despite many years of research, the genetic background of hypertrophic cardiomyopathy is not fully understood. However, even the rapidly accumulating next-generation sequencing data and the accompanying computer analysis used to predict the effects of the detected variants on the protein structure do not answer the existing questions. Therefore, analysis of the segregation and 3D structures of the full-length protein is still relevant to prove or exclude the pathogenicity of the identified variants.

In this study, we analyzed the effect of the rs749628307 variant in the VCL gene leading to the amino acid substitution p.Arg230His on the protein structure and clinical features of hypertrophic cardiomyopathy in carriers of the pathogenic variant rs397516037 in the MYBPC3 gene. Our analysis showed that the rs749628307 variant in the VCL gene is likely to neither significantly alter the protein structure nor affect the severity and form of clinical features of hypertrophic cardiomyopathy.

Consent for publication

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

Funding

This study was supported by the Russian Science Foundation (grant # 22-15-00243).

No conflict of interest is reported.

The article was received on 14/12/2021

ISSN 0022-9040. Kardiologiia. 2023;63(3). DOI: 10.18087/cardio.2023.3.n1937

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