ISSN 1560-4071 (print)
ISSN 2618-7620 (online)

РОССИЙСКИЙ КАРДИОЛОГИЧЕСКИЙ ЖУРНАЛ

Russian Journal of Cardiology

SCIENTIFIC, PEER-REVIEWED MEDICAL JOURNAL

RUSSIAN SOCIETY OF CARDIOLOGY

IN ISSUE:

IN FOCUS:
Genetics in cardiology

№ 10 | 2020

РОССИЙСКОЕ КАРДИОЛОГИЧЕСКОЕ ОБЩЕСТВО

Russian Society of Cardiology

Scientific peer-reviewed medical journal

Mass media registration certificate № 017388 dated 06.04.1998

Periodicity — 12 issues per year
Circulation — 7 000 copies

The Journal is in the List of the leading scientific journals and publications of the Supreme Examination Board (VAK)

The Journal is included in Scopus, EBSCO, DOAJ

Russian Citation Index:
SCIENCE INDEX (2018) 3,054
Impact-factor (2018) 1,082

Complete versions of all issues are published: *www.elibrary.ru*

Instructions for authors: *https://russjcardiol.elpub.ru/jour/about/submissions#authorGuidelines*

Submit a manuscript: *www.russjcardiol.elpub.ru*

Subscription: *www.roscardio.ru/ru/subscription.html*

Open Access

Printed: OneBook, Sam Poligraphist, Ltd. 129090, Moscow, Protopopovsky per., 6. www.onebook.ru

Font's license №180397 от 21.03.2018

RUSSIAN JOURNAL OF CARDIOLOGY

№ 25 (10) 2020

founded in 1996

Editorial office:
119049, Moscow,
ul. Shabolovka, 23-254
e-mail: cardiojournal@yandex.ru
Tel. +7 (985) 768 43 18

Publisher:
Silicea-Poligraf
e-mail: cardio.nauka@yandex.ru

https://russjcardiol.elpub.ru
doi:10.15829/1560-4071-2020-10

ISSN 1560-4071 (print)
ISSN 2618-7620 (online)

CONTENTS

ORIGINAL ARTICLES

CLINICAL OBSERVATION

https://russjcardiol.elpub.ru
doi:10.15829/1560-4071-2020-4098

ISSN 1560-4071 (print)
ISSN 2618-7620 (online)

Cardiometabolic risk factors and their relationship with the interleukin-6 receptor gene polymorphism (rs2228145) in patients with hypertrophic cardiomyopathy

Bezhanishvili T. G.[1], Gudkova A. Ya.[1,2], Davydova V. G.[2], Andreeva S. E.[2], Krutikov A. N.[2], Semernin E. N.[1,2], Kudryavtsev B. N.[1], Pyko S. A.[3], Kostareva A. A.[1,2], Shlyakhto E. V.[1,2]

Aim. To analyze associations of interleukin-6 receptor gene (*IL6R*) polymorphism (rs2228145) with the clinical course characteristics of hypertrophic cardiomyopathy (HCM) in groups of patients with various cardiometabolic risk factors.
Material and methods. The sample consisted of 123 patients with HCM. The age of the included patients ranged from 18 to 91 years (59 [41; 66,5]), of whom 59 were men, 64 — women. Two age groups were identified: the first group included patients from 18 to 44 years old, the second — 45 years and older. The control group consisted of 200 people without cardiovascular diseases and other severe comorbidities.
For genetic testing, DNA was isolated from peripheral blood lymphocytes. Genotyping of the *IL6R* gene polymorphism (rs2228145) was carried out by real-time polymerase chain reaction.
Results. A significant prevalence of CC genotype of the *IL6R* gene polymorphism (rs2228145) was revealed in patients aged ≥45 years compared with the control group, which occurred in 14,1% and 3,0% of cases, respectively (CC:AC+AA, odds ratio (OR), 0,885, 95% confidence interval (CI), 1,051-0,691, p=0,006), and insignificant prevalence of C allele in this group, which does not reach the level of significance (A:C, OR, 0,870, 95% CI, 0,427-1,02, p=0,06). The prevalence of CC genotype (15,1% vs 3,0%) and C allele (39,0% vs 29,0%) was revealed in patients with HCM in combination with hypertension (HTN) compared with the control group (CC:AS+AA, OR=0,174, 95% CI, 0,047-0,650), p=0,004); (A:C, OR=0,638, 95% CI, 0,406-1,002), p=0,05).
Conclusion. The relationship between the *IL6R* gene polymorphism (rs2228145) and HTN in patients with HCM was confirmed. The presence of CC genotype and C allele of the rs2228145 polymorphism is significantly more common in patients with HCM with the disease onset ≥45 years of age. The presence of CC genotype and C allele of the *IL6R* gene polymorphism (rs2228145) is associated with HTN in patients with HCM.

Key words: hypertrophic cardiomyopathy, interleukin-6, interleukin-6 receptor gene, hypertension.

[1]First Pavlov State Medical University of St. Petersburg, St. Petersburg; [2]Almazov National Medical Research Center, St. Petersburg; [3]Saint Petersburg Electrotechnical University "LETI", Saint Petersburg, Russia.

Relationships and Activities: none.

Bezhanishvili T. G.* ORCID: 0000-0002-3167-6340, Gudkova A. Ya. ORCID: 0000-0003-0156-8821, Davydova V. G. ORCID: 0000-0002-0233-5555, Andreeva S. E. ORCID: 0000-0001-7306-9525, Krutikov A. N. ORCID: 0000-0001-6897-6171, Semernin E. N. ORCID: 0000-0001-8447-6919, Kudryavtsev B. N. ORCID: 0000-0002-1236-822X, Pyko S. A. ORCID: 0000-0001-6625-3770, Kostareva A. A. ORCID: 0000-0002-9349-6257, Shlyakhto E. V. ORCID: 0000-0003-2929-0980.

*Corresponding author:
tinatin93@rambler.ru

Received: 07.09.2020
Revision Received: 24.09.2020
Accepted: 30.09.2020

For citation: Bezhanishvili T. G., Gudkova A. Ya., Davydova V. G., Andreeva S. E., Krutikov A. N., Semernin E. N., Kudryavtsev B. N., Pyko S. A., Kostareva A. A., Shlyakhto E. V. Cardiometabolic risk factors and their relationship with the interleukin-6 receptor gene polymorphism (rs2228145) in patients with hypertrophic cardiomyopathy. *Russian Journal of Cardiology*. 2020;25(10):4098. (In Russ.) doi:10.15829/1560-4071-2020-4098

Hypertrophic cardiomyopathy (HCM) is the most common inherited heart disease with an autosomal dominant manner of inheritance without a clear geographical, ethnic and sex distribution structure, which can manifest at any age [1]. In recent years, HCM pathogenesis are considered from the standpoint of the contribution of unmodifiable determinants (causal mutations, age, sex) and potentially modifiable factors (comorbidity). According to the meta-analysis reported by Finnochiaro G, et al. (2017), the debut and features of HCM in adults under 45 years of age is largely determined by causal genetic variants, severe myocardial hypertrophy (hypertrophy >3 cm) and a high risk of sudden death [2]. At the same time, the onset of the disease in older age groups often occurs under the influence of comorbidities, especially cardiometabolic risk factors, the frequency of which significantly increases with age [3]. Features of HCM course in older age groups are also associated with the presence of coronary artery disease in patients, the debut of which falls at this age [3, 4]. The urgency of comorbidity in older patients with HCM is specified by the high frequency of hypertension (HTN) and obesity [5-8]. According to Olivotto I, et al. (2013), only 25% of patients with HCM had normal body weight, 38% — overweight, 37% — OB, 27% — HET, 5% — type 2 diabetes (T2D) [5]. There are studies on the influence of obesity on myocardial remodeling in HCM [9, 10].

The importance of systemic inflammation in the pathogenesis of metabolic syndrome (MS) and its components has now been demonstrated in many studies [11-14]. The role of serum cytokines, in particular interleukin-6 (IL-6), has been shown in the pathogenesis of various cardiovascular diseases [15-20]. Thus, in the study by Buzas K, et al. (2004) revealed an increase in the concentration of IL-6 and soluble IL-6 receptor in the blood of patients with HCM [21].

IL-6 is one of the proteins of paracrine signaling, which is secreted during inflammation and has a diverse action, participating not only in inflammation, but also in the regulation of the endocrine functions and metabolism [22]. The broad spectrum of action of IL-6 is due to its transmembrane receptors, which are located in most body tissues: myocardium, spleen, kidneys, lungs, liver and brain. IL-6 receptors are not directly involved in signal transduction, but they provide homodimerization of another transmembrane receptor (gp130), which triggers the intracellular signaling [22]. The IL-6 receptor also exists in a soluble form and is the extracellular domain of the membrane receptor. Through the effects of soluble IL-6 receptor, gp130 is activated even in those cells that do not have the membrane IL-6 receptor [23]. In particular, IL-6 can cause myocardial hypertrophy by acting on gp130, despite the fact that there are no IL-6 receptors in cardiomyocytes [24].

Compared with studies concerning the plasma IL-6 level, papers devoted to polymorphism of IL6 receptor (*IL6R*) gene and their relationship with clinical and paraclinical parameters are much less common in the literature and concern mainly coronary artery disease [25-27]. A few works on the frequency of polymorphic variants of the *IL6R* gene are devoted to MS [28, 29], HTN [30], dyslipidemia [31, 32] and aortic stenosis [33]. The distribution of the polymorphic variant rs2228145 of the *IL6R* gene and its relationship with the manifestations of HCM are practically not studied to date. The aim of this study was to study the association of *IL6R* gene polymorphism rs2228145 with the clinical course of HCM.

Material and methods

The study group consisted of 123 patients with HCM. The diagnosis was made based on the 2014 ESC guidelines on diagnosis and management of HCM [1]. The age of the patients included in the study ranged from 18 to 91 years (59 [41; 66.5]), There were 59 men and 64 women. Two age groups were identified: the first group included patients aged 18-44 years, the second — patients aged ⩾45 years. Criteria for HCM with concomitant hypertension were as follows:

1. Positive family history of HCM or sudden cardiac death (SCD) at a young age in first-degree relatives.

2. Discrepancy between severe left ventricular hypertrophy (maximum wall thickness ⩾15 mm) and recent mild to moderate HTN with adequate medical adherence, as well as the absence of other causes of left ventricular hypertrophy.

The control group consisted of 200 people without cardiovascular diseases and other severe comorbidities. All patients signed informed consent. For genetic testing, DNA was isolated from peripheral blood lymphocytes. Genotyping of *IL6R* gene polymorphism rs2228145 was carried out by real-time polymerase chain reaction.

Statistical processing was carried out using Microsoft Excel 2010, IBM SPSS and Jamovi packages. Normal distributed data were presented as mean ± standard deviation. Non-normally distributed data are presented as median and quartiles. For analysis of relationship of qualitative variables, contingency tables were constructed. To compare frequencies, we used the Pearson's chi-squared test. If the expected frequency in contingency tables was <5, then the exact test was used. For contingency tables 2x2 with a small number of objects (up to 40-50), the chi-

Table 1
Clinical characteristics of patients with HCM, the incidence of comorbidities and cardiometabolic risk factors

Parameter	HCM (n=123), n (%)
Sex	
Male	59 (48%)
Female	64 (52%)
Age	
<45 years old	38 (30,9%)
⩾45 years old	85 (69,1%)
Class I-II HF	88 (71,5%)
Class III-IV HF	35 (28,5%)
Atrial fibrillation	31 (25,2%)
Ventricular tachycardia	7 (5,7%)
Grade V ventricular premature beats	16 (13,0%)
Blocks	
Atrioventricular block	1 (0,8%)
Sinoatrial block	4 (3,3%)
LVOTO	49 (39,8%)
SCD	3 (2,4%)
Hypertension	73 (59,3%)
Obesity (BMI >30)	45 (36,6%)
Type 2 diabetes	24 (19,5%)

Abbreviations: LVOTO — left ventricular outflow tract obstruction, SCD — sudden cardiac death, HCM — hypertrophic cardiomyopathy, BMI — body mass index, HF — heart failure.

Table 2
Echocardiographic parameters in patients with HCM in different age groups

Parameter	<45 years (n=38) Me [25%;75%]	⩾45 years (n=85) Me [25%;75%]	p
Age, years	26 [18,5;39,8]	63 [58;72]	<0,001
IVS, mm	22 [19;25]	20 [18;22]	0,016
LVPW, mm	12 [10;17]	13 [11;15]	0,381
LA, mm	42,5 [38,3;50]	47 [43;52]	0,019
LA index	26 [22;29]	25 [23;28]	0,982
MMI	202 [153;259]	176 [150;215]	0,143
EDD, mm	42 [37;50]	48 [43;51]	0,015
EDD index	25 [20,5;27]	25 [23;28]	0,236
EF, %	64,5 [60;71,5]	63 [57;68]	0,194

Abbreviations: LVPW — left ventricular posterior wall at diastole, MII — myocardial mass index, EDD — end-diastolic dimension, LA — left atrium, IVS — interventricular septum at diastole, EF — ejection fraction.

squared test was used with a correction for continuity, and if its application were violated, Fisher's exact test was used. Differences were considered significant at p<0,05.

Results

Clinical characteristics of patients. The experimental group in the study consisted of patients with HCM aged 18 to 91 years (59 [41; 66.5]; men — 48%, women — 52%). The most frequent comorbidity was HTN, which occurred in 59,3% of cases and was recorded mainly in patients aged ⩾45 years (94,5%). HTN was observed in 81,2% of patients aged ⩾45 years, while among patients aged <45 years it was detected in 10,5% of cases (p<0,001). Obesity was detected in 50,6% of older patients and in 5,3% of patients in the younger age group (p<0,001). T2D was diagnosed in 27,1% of patients aged ⩾45 years and in 2,6% of patients aged <45 years (p=0,002). Atrial fibrillation occurred in 29,4% of patients in the older age group and in 2,6% of patients aged <45 years (p=0,108). Ventricular arrhythmias were detected in 10,5% of patients in the younger age group, while in the older age group they were detected in 3,5% (p=0,122). In young patients, atrioventricular block was detected in 2,6% of cases and sinoatrial block in 5,3%, while in the older age group they were 0% and 2,4%, respectively (p=0,223). Left ventricular outflow tract obstruction (LVOTO) was detected in half of young patients and in 35,3% of patients in the older age group. Thus, the presence of comorbidities, in particular cardiometabolic risk factors, is most typical for patients aged ⩾45 years. Young patients showed a predominance of arrhythmias and conduction disorders and a high incidence of obstructive HCM. SCD was recorded with approximately the same frequency in patients of both age groups (2,4% vs 2,6%).

The clinical characteristics of the patients included in the study are presented in Table 1.

The interventricular septal (IVS) thickness at diastole in patients with HTN ranged from 12 to 28 mm (20 [18; 22]) and did not statistically differ from that in non-HTN patients, in whom the IVS thickness varied from 13 to 45 mm (22 [18; 25]) (p=0,144). However, there was a significant thickening of the left ventricular posterior wall (LVPW) at diastole in HTN patients (13 [12; 16] and 11 [10; 16], p=0,029). The end-diastolic dimension (EDD) in patients with HTN was significantly greater than in non-HTN patients (49 [43; 52] and 44 [38; 49], p=0,004). The left atrial (LA) diameter was higher in patients with HCM and HTN compared to patients with HCM without HTN (47 [43; 53] and 44 [39,3; 50], p=0,007).

It should be taken into account that among patients with HCM there were isolated cases of

Table 3

Age characteristics of the distribution of genotypes and alleles of IL6R gene polymorphism rs2228145 in patients with HCM

	⩾45 years (n=85)	Control (n=200)	p
AA	32 (37,6%)	90 (45,0%)	0,021
AC	41 (48,2%)	104 (52,0%)	
CC	12 (14,1%)	6 (3,0%)	
A	105 (61,8%)	284 (71,0%)	0,06
C	65 (38,2%)	116 (29,0%)	

Table 4

Distribution of genotypes and alleles of IL6R gene polymorphism rs2228145 in the group of patients with HCM in combination with HTN and in the control group

	HCM and HNT (n=73)	Control (n=200)	P
AA	27 (37,0%)	90 (45,0%)	0,015
AC	35 (47,9%)	104 (52,0%)	
CC	11 (15,1%)	6 (3,0%)	
A	89 (61,0%)	284 (71,0%)	0,05
C	57 (39,0%)	116 (29,0%)	

Abbreviations: HTN — hypertension, HCM — hypertrophic cardiomyopathy.

Table 5

Distribution of genotypes and alleles of IL6R gene polymorphism rs2228145 in obese patients with HCM and in the control group

	HCM and obesity (n=45)	Control (n=200)	P
AA	18 (40,0%)	90 (45,0%)	0,058
AC	21 (46,7%)	104 (52,0%)	
CC	6 (13,3%)	6 (3,0%)	
	(n=90)	(n=200)	
A	57 (63,3%)	284 (71,0%)	0,193
C	33 (36,7%)	116 (29,0%)	

Abbreviation: HCM — hypertrophic cardiomyopathy.

dilated phase of the disease. In this case the diagnosis was verified based on a family history of HCM and confirmed by the presence of mutations in the genes of myocardial contractile proteins.

Patients with normal body weight were found to have more pronounced IVS hypertrophy compared with obese patients (21 [18,3; 25,0] and 20 [17; 21], respectively, p=0,042). On the contrary, there was a higher LVPW thickness (14 [12; 16] and 12 [10; 14], respectively, p<0,001) and LA diameter (48 [43; 54] and 46 [41.3; 50], respectively, p=0,037) in obese patients compared with patients with normal body weight.

It was found that the IVS thickness in the group of patients <45 years old prevails in comparison with the group of patients aged ⩾45 years (22 [19; 25] vs 20 [18; 22], p=0,016). In patients aged ⩾45 years, compared with patients aged <45 years, there was a higher LA diameter (47 [43; 52] and 42.5 [38,3; 50], respectively, p=0,019) and EDD (48 [43; 51] and 42 [37; 50], p=0,015). Obstructive HCM occurred in 35,3% of patients aged ⩾45 years and in half of patients aged <45 years (Table 2).

For obese patients with HTN, there were significantly higher values of LVPW thickness compared with patients with normal body weight and without HTN (145 [12,3; 16] and 11 [10; 15,8], respectively, p=0,002). There were higher LA diameter (47,5 [43; 53,8] vs 43 [39; 49,5], p=0,008) and EDD (48,5 [43,3; 52] vs 43,5 [38; 49], p=0,012) in patients with HTN and obesity compared with the group of non-obese patients without HTN.

Distribution of genotypes and alleles of the *IL6R* gene polymorphism rs2228145 in patients with HCM. A significant prevalence of the CC genotype was revealed in patients with HCM aged ⩾45 years compared with the control group, which occurred in 14,1% and 3,0% of cases, respectively (CC:AC+AA, odds ratio (OR), 0,885, 95% confidence interval (CI), 1,051-0,691, p=0,006). There was also prevalence of C allele in this group, which does not reach the level of significance (A:C, OR, 0,870, 95% CI, 0,427-1,02, p=0,06) (Table 3).

The prevalence of CC genotype (15,1% vs 3,0%) and C allele (39,0% vs 29,0%) was revealed in patients with HCM in combination with HTN compared with the control group (CC:AC+AA, OR=0,174, 95% CI, 0,047-0,650, p=0,004); (A:C, OR, 0,638, 95% CI, 0,406-1,002, p=0,05) (Table 4).

There were no significant differences in the distribution of genotypes and the occurrence of alleles of *IL6R* gene polymorphism rs2228145 in obese patients with HCM (Table 5).

There were no significant differences in echocardiography in patients with HCM, depending on the *IL6R* gene polymorphism rs2228145.

Discussion

The clinical course of the disease is significantly influenced by comorbidities, the incidence of which increases with age [3, 5-8, 34, 35]. In the experimental group of patients with HCM, the incidence of HTN, obesity, T2D, atrial fibrillation was higher

in patients with HCM in the older age group than in young patients, which is consistent with the data of most studies [5, 36]. The study by Ingles J, et al. (2017) and numerous other works revealed the higher prevalence HTN in older patients with non-familial HCM [3, 5-8, 34, 35, 37]. Age of onset is an important determinant of the clinical course of HCM [2, 38-41]. As a result of the study, we revealed an association of the CC genotype and C allele of *IL6R* gene polymorphism rs2228145 with HTN in patients with HCM with the onset of disease ⩾45 years. Our results are consistent with the work of LV Topchieva et al. (2020), which also revealed a relationship between the development of AH and the carriage of the CC genotype in the *IL6R* gene [30]. As you know, HTN is accompanied by an increase in proinflammatory cytokines in plasma and vascular tissues, including IL-6 [14]. In our work, no differences were found in the distribution of genotypes and alleles of the *IL6R* gene polymorphism rs2228145 in obese and non-obese patients. A reliable analysis of the distribution of genotypes and alleles in the group of obese patients with HCM and HTN was not possible due to the small the number of patients. However, Jiang CQ, et al. showed that in carriers of the AA genotype of the *IL6R* gene polymorphism rs2228145, MS was more common, despite a lower level of plasma IL-6. In white carriers of the same genotype, central obesity was more common and this phenomenon did not apply to Mongoloid people. At the same time, the CC genotype was associated with a higher level of IL-6, but this was not accompanied by a high incidence of HTN [29]. The role of serum IL-6 level and *IL6R* gene polymorphisms in the pathogenesis of HCM in patients with obesity and HTN requires further clarification. It is possible that this depends on the patient's age and are modulated by various components of MS.

Conclusion

The relationship between the *IL6R* gene polymorphism (rs2228145) and HTN in patients with HCM was confirmed. However, its influence on myocardial remodeling, hypertrophy, and LA parameters require additional studies involving a larger number of patients. The presence of CC genotype and C allele of the *IL6R* gene rs2228145 polymorphism is significantly more common in patients with HCM with the disease onset ⩾45 years of age. The presence of CC genotype and C allele of the *IL6R* gene polymorphism (rs2228145) is associated with HTN in patients with HCM. Further analysis of the relationship between serum IL-6 levels and polymorphic variants, as well as their effect on the clinical course of HCM in different age groups, requires further research.

Relationships and Activities: none.

References

1. Elliot P, Zamorano JL, Anastasakis A, et al. 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: The task force for the diagnosis and management of hypertrophic cardiomyopathy of the European Society of Cardiology (ESC). Eur Heart J. 2014;35(39):2733-79. doi:10.1093/eurheartj/ehu284.
2. Finocchiaro G, Magavern E, Sinagra G, et al. Impact of demographic features, lifestyle, and comorbidities on the clinical expression of hypertrophic cardiomyopathy. J Am Heart Assoc. 2017;6(12):e007161. doi:10.1161/JAHA.117.007161.
3. Maron BJ, Rowin EJ, Casey SA, et al. Risk stratification and outcome of patients with hypertrophic cardiomyopathy ⩾60 years of age. Circulation. 2013;127(5):585-93. doi:10.1161/CIRCULATIONAHA.112.136085.
4. Kaplunova VYu, Shakaryants GA, Kozhevnikova MV, et al. Hypertrophic Cardiomyopathy and Ischemic Heart Disease. Variants of Combination Pathology. Kardiologiia. 2017;57(12):16-24. (In Russ.) doi:10.18087/cardio.2017.12.10062.
5. Olivotto I, Maron BJ, Tomberli B, et al. Obesity and its association to phenotype and clinical course in hypertrophic cardiomyopathy. J Am Coll Cardiol. 2013;62(5):449-57. doi:10.1016/j.jacc.2013.03.062.
6. Topol EJ, Traill TA, Fortuin NJ. Hypertensive Hypertrophic Cardiomyopathy of the Elderly. N Engl J Med. 1985;312(5):277-83. doi:10.1056/NEJM198501313120504.
7. Karam R, Lever HM, Healy BP. Hypertensive hypertrophic cardiomyopathy or hypertrophic cardiomyopathy with hypertension? A study of 78 patients. J Am Coll Cardiol. 1989;13(3):580-4. doi:10.1016/0735-1097(89)90596-2.
8. Shimizu M, Sugihara N, Sffimizu K, et al. Asymmetrical septal hypertrophy in patients with hypertension: A type of hypertensive left ventricular hypertrophy or hypertrophic cardiomyopathy combined with hypertension? Clin Cardiol. 1993;16(1):41-6. doi:10.1002/clc.4960160110.
9. Canepa M, Sorensen LL, Pozios I, et al. Comparison of clinical presentation, left ventricular morphology, hemodynamics, and exercise tolerance in obese versus nonobese patients with hypertrophic cardiomyopathy. Am J Cardiol. 2013;112(8):1182-9. doi:10.1016/j.amjcard.2013.05.070.
10. Ommen SR, Lopez-Jimenez F. Obesity and hypertrophic cardiomyopathy: Chickens, eggs, and causality: Clinical skills remain the key to caring for patients. J Am Coll Cardiol. 2013;62(5):458-9. doi:10.1016/j.jacc.2013.03.063.
11. Ross R. Atherosclerosis — An inflammatory disease. New England Journal of Medicine. 1999;340(2):115-26. doi:10.1056/NEJM199901143400207.
12. Vostrikova NV, Fyodorov DV, Klimova EE, Bishevsky KM. Prognostic importance of inflammation markers (c-reactive protein and interleukin-6) in patients with arterial hypertension. Bulletin of Medical Science. 2019;1(13):44-6. (In Russ.) doi:10.31684/2541-8475.2019.1(13).44-46.
13. Harrison DG, Guzik TJ, Lob HE, et al. Inflammation, immunity, and hypertension. Hypertension. 2011;57(2):132-40. doi:10.1161/HYPERTENSIONAHA.110.163576.
14. Bautista LE, Vera LM, Arenas IA, et al. Independent association between inflammatory markers (C-reactive protein, interleukin-6, and TNF-α) and essential hypertension. J Hum Hypertens. 2005;19(2):149-54. doi:10.1038/sj.jhh.1001785.
15. Vostrikova NV, Fyodorov DV, Mamayev AN, et al. Markers of inflammatory reaction (c-reactive protein and interleukin-6) in arterial hypertension. Sibirskii meditsinskii jurnal (Tomsk). 2009;24(4-1):33-4. (In Russ.)
16. Osipova OA, Suyazova SB, Vlasenko MA, et al. Role of proinflammatory cytokines in chronic heart failure. Rossiiskii mediko-biologicheskii vestnik imeni akademika I. P. Pavlova. 2013;21(2):130-5. (In Russ.) doi:10.17816/PAVLOVJ20132130-135.
17. Kosmala W, Derzhko R, Przewlocka-Kosmala M, et al. Plasma levels of TNF-α, IL-6, and IL-10 and their relationship with left ventricular diastolic function in patients with stable angina pectoris and preserved left ventricular systolic performance. Coron Artery Dis. 2008;19(6):375-82. doi:10.1097/MCA.0b013e3282fc617c.
18. Hedayat M, Mahmoudi MJ, Rose NR, et al. Proinflammatory cytokines in heart failure: Double-edged swords. Heart Failure Reviews. 2010;15:543-62. doi:10.1007/s10741-010-9168-4.
19. Gabriele P, Zhi Fang S, Tonny DT, et al. Activation of the cardiac interleukin-6 system in advanced heart failure. Eur J Heart Fail. 2001;3(4):415-21. doi:10.1016/S1388-9842(01)00137-4.
20. Popkova TV, Novikov DS, Nasonov EL. Interleykin 6 i serdechno-sosudistaya patologiya pri revmatoidnom artrite. Rheumatology Science and Practice. 2011;49(4):64-72. (In Russ.) doi:10.14412/1995-4484-2011-63.
21. Buzás K, Megyeri K, Hõgye M, et al. Comparative study of the roles of cytokines and apoptosis in dilated and hypertrophic cardiomyopathies. Eur Cytokine Netw. 2004;15(1):53-9.
22. Kishimoto T, Akira S, Narazaki M, et al. Interleukin-6 family of cytokines and gp130. Blood. 1995;86(4):1243-54. doi:10.1182/blood.V86.4.1243.bloodjournal8641243.
23. Kishimoto T, Taga T, Akira S. Cytokine signal transduction. Cell. 1994;76:253-62. doi:10.1016/0092-8674(94)90333-6.
24. Hirota H, Yoshida K, Kishimoto T, et al. Continuous activation of gp130, a signal-transducing receptor component for interleukin 6-related cytokines, causes myocardial hypertrophy in mice. Proc Natl Acad Sci U S A. 1995;92(11):4862-6. doi:10.1073/pnas.92.11.4862.
25. Chen Z, Qian Q, Tang C, et al. Association of two variants in the interleukin-6 receptor gene and premature coronary heart disease in a Chinese Han population. Mol Biol Rep. 2013;40(2):1021-6. doi:10.1007/s11033-012-2143-5.
26. Zhou J, Chen X, Ye H, et al. An Association Study between Genetic Polymorphism in the Interleukin-6 Receptor Gene and Coronary Heart Disease. Biomed Res Int. 2014;504727. doi:10.1155/2014/504727.
27. Mitrokhin V, Nikitin A, Brovkina O, et al. Association between interleukin-6/6R gene polymorphisms and coronary artery disease in Russian population: Influence of interleukin-6/6R gene polymorphisms on inflammatory markers. J Inflamm Res. 2017;10:151-60. doi:10.2147/JIR.S141682.
28. Esteve E, Villuendas G, Mallolas J, et al. Polymorphisms in the interleukin-6 receptor gene are associated with body mass index and with characteristics of the metabolic syndrome. Clin Endocrinol (Oxf). 2006;65(1):88-91. doi:10.1111/j.1365-2265.2006.02553.x.
29. Jiang CQ, Lam TH, Liu B, et al. Interleukin-6 Receptor Gene Polymorphism Modulates Interleukin-6 Levels and the Metabolic Syndrome: GBCS-CVD. Obesity. 2010;18(10):1969-74. doi:10.1038/oby.2010.31.
30. Topchieva LV, Korneva VA, Kurbatova IV. The relationship of the carriership of allelic variations in rs2228145 (A>C) of the IL6R gene with the levels of VCAM1 and ICAM1 gene transcripts in patients with essential hypertension. Vavilovskii Zhurnal Genetiki i Selektsii. 2020;24(1):96-101. (In Russ.) doi:10.18699/VJ20.600.
31. Chu NF, Lin FH, Chin HC, et al. Association between interleukin-6 receptor gene variations and atherosclerotic lipid profiles among young adolescents in Taiwan. Lipids Health Dis. 2011;10:136. doi:10.1186/1476-511X-10-136.
32. Arguinano AA, Naderi E, Ndiaye NC, et al. IL6R haplotype rs4845625*T/rs4537545*C is a risk factor for simultaneously high CRP, LDL and ApoB levels. Genes Immun. 2017;18(3):163-9. doi:10.1038/gene.2017.16.
33. Wypasek E, Potaczek DP, Lamplmayr M, et al. Interleukin-6 receptor ASP358ALA gene polymorphism is associated with plasma C-reactive protein levels and severity of aortic valve stenosis. Clin Chem Lab Med. 2014;52(7):1049-56. doi:10.1515/cclm-2013-0606.
34. Abel ED, Litwin SE, Sweeney G. Cardiac remodeling in obesity. Physiological Reviews. 2008;88:389-419. doi:10.1152/physrev.00017.2007.
35. Poliakova AA, Gudkova AY, Krutikov AN, et al. Hypertrophic cardiomyopathy in the older age group: The effect of cardiometa-

bolic risk factors and rs2290149 and rs10838692 of the MADD gene. Arter Hypertens. 2018;24(1):29-40. (In Russ.) doi:10.18705/1607-419X-2018-24-1-29-40.
36. Poliakova AA, Baranova EI, Semernin EN, et al. Gender differences of clinical manifestation and cardiac remodeling in idiopathic hypertrophic cardiomyopathy in elderly patients. Russ J Cardiol. 2018;154(2):13-8. (In Russ.) doi:10.15829/1560-4071-2018-2-13-18.
37. Ingles J, Burns C, Bagnall RD, et al. Nonfamilial Hypertrophic Cardiomyopathy: Prevalence, Natural History, and Clinical Implications. Circ Cardiovasc Genet. 2017;10(2):e001620. doi:10.1161/CIRCGENETICS.116.001620.
38. Maron BJ, Piccininno M, Casey SA, et al. Relation of extreme left ventricular hypertrophy to age in hypertrophic cardiomyopathy. Am J Cardiol. 2003;91(5):626-8. doi:10.1016/S0002-9149(02)03326-X.
39. Gudkova AYa, Davidova VG, Bezhanishvili TG, et al. Characterisation of circulating microRNA-21 levels in patients with hypertrophic cardiomyopathy. Therapeutic Archive. 2020;92(4):51-6. (In Russ.) doi:10.26442/00403660.2020.04.000272.
40. Spirito P, Maron BJ. Relation Between Extent of Left Ventricular Hypertrophy Hypertrophic Cardiomyopathy. JACC. 1990;15(4):808-13. doi:10.1016/0735-1097(90)90278-W.
41. Maron B, Piccininno M, Casey S, et al. Relation of extreme left ventricular hypertrophy to age in hypertrophic cardiomyopathy. Am J Cardiol. 2003;91(5):626-8. doi:10.1016/S0002-9149(02)03326-X.
42. Wolf J, Rose-John S, Garbers C. Interleukin-6 and its receptors: a highly regulated and dynamic system. Cytokine. 2014;70:11-20. doi:10.1016/j.cyto.2014.05.024.

https://russjcardiol.elpub.ru
doi:10.15829/1560-4071-2020-3880

ISSN 1560-4071 (print)
ISSN 2618-7620 (online)

Next generation sequencing in sudden cardiac death (pilot study)

Maksimov V. N.[1,2], Ivanoshchuk D. E.[1], Orlov P. S.[1], Ivanova A. A.[1], Malyutina S. K.[1,2], Maksimova S. V.[2], Rodina I. A.[3], Khamovich O. V.[3], Novoselov V. P.[2,3], Voevoda M. I.[1,2]

Aim. To search for causal mutations in candidate genes responsible for the development of sudden cardiac death (SCD) in men who died under the age of 45.

Material and methods. The SCD group (n=37) was formed using the criteria the World Health Organization and the European Society of Cardiology. Autopsy material was collected from men who died suddenly outside medical institutions and underwent forensic medical examination according to the standard protocol. Autopsy revealed no morphological changes that could explain sudden death. The mean age was 32,4±6,4 years. Genomic DNA was isolated from myocardial tissue using phenol-chloroform extraction. Clinical exome sequencing was performed. At first, we analyzed the results of sequencing of 24 genes, mutations in which lead to cardiovascular diseases associated with an increased risk of SCD: *KCNQ1, KCNH2, SCN5A, AKAP9, ANK2, CACNA1C, CALM1, CALM2, CAV3, KCNE1, KNCJNE2, KCNE2, SCN4B, SNTA1, MYH2, APOB, KCNA5, TGFB3, NEB, PDX1, FLNC, PLEC, KCND3.*

Results. Of 37 samples, we revealed 13 probable pathogenic missense mutations in 9 samples (24,3%). Of 13 probable pathogenic variants, 5 were new.

Conclusion. This pilot study provides following conclusions: it is necessary to continue molecular autopsy research in Russia; to increase the effectiveness of detecting causal mutations; to reduce the age of patients with SCD included in the study; studying the families of deceased; cooperation of experienced specialists — forensic pathologist, laboratory geneticist, cardiologist.

Key words: sudden cardiac death, mutation, NGS, gene panel, exome, molecular autopsy.

Relationships and Activities. This paper was supported by the Russian Foundation for Basic Research grant № 17-29-06026, partially by the grant № NSh-2595.2020.7, and budget projects № 0324-2016-0002, 0120.0502961.

[1]Research Institute of Therapy and Preventive Medicine, Federal Research Center Institute of Cytology and Genetics, Novosibirsk; [2]Novosibirsk State Medical University, Novosibirsk; [3]Novosibirsk Regional Clinical Bureau of Forensic Medicine, Novosibirsk, Russia.

Maksimov V. N.* ORCID: 0000-0002-7165-4496, Ivanoshchuk D. E. ORCID: 0000-0002-0403-545X, Orlov P. S. ORCID: 0000-0001-9371-2178, Ivanova A. A. ORCID: 0000-0002-9460-6294, Malyutina S. K. ORCID: 0000-0001-6539-0466, Maksimova S. V. ORCID: 0000-0002-2472-181X, Rodina I. A. ORCID: 0000-0003-2799-0756, Khamovich O. V. ORCID: 0000-0002-2960-193X, Novoselov V. P. ORCID: 0000-0002-6312-5543, Voevoda M. I. ORCID: 0000-0001-9425-413X.

*Corresponding author: medik11@mail.ru

Received: 04.05.2020
Revision Received: 22.06.2020
Accepted: 09.08.2020

For citation: Maksimov V. N., Ivanoshchuk D. E., Orlov P. S., Ivanova A. A., Malyutina S. K., Maksimova S. V., Rodina I. A., Khamovich O. V., Novoselov V. P., Voevoda M. I. Next generation sequencing in sudden cardiac death (pilot study). *Russian Journal of Cardiology*. 2020;25(10):3880. (In Russ.) doi:10.15829/1560-4071-2020-3880

Sudden cardiac death (SCD) is one of the important unsolved health issues. Current trends in medicine are associated with the widespread introduction of personalized preventive strategies aimed at correcting risk factors for pathology and conducting primary prevention, which contributes to reducing morbidity and mortality [1, 2]. In the United States, more than 220 thousand people die from SCD every year [3]. Sudden deaths are known to account for 20% of total mortality and 50% of cardiovascular mortality in Western countries [1]. In Russia, National guidelines for risk assessment and prevention of SCD was developed, where most of them are recommendations for the correction of risk factors and the prevention of SCD, but they all relate to individuals with known cardiovascular disease [4, 5]. The risk of SCD is the highest in persons who have suffered cardiac arrest, myocardial infarction or have a history of heart failure, but up to 80% of SCD occur in patients with asymptomatic course of cardiovascular disease [6]. SCD is one of the most important unresolved problems in forensic practice due to the inability to accurately determine the cause of sudden death. In the past three decades, causative genes for inherited arrhythmias have been successfully identified. At the same time, it became obvious that the genetic architecture of this pathological phenotype is more complex than previously thought [2, 7]. Currently, the impact of genetics and genetic testing on the clinical management of patients with these diseases is not in doubt. In particular, genetic tests are an important tool for identifying presymptomatic individuals carrying a genetic variant that predisposes them to SCD. High-throughput sequencing technologies offer new opportunities for deeper study of the genetic background underlying these deadly diseases and early detection of individuals at risk of SCD [1, 8-10]. Molecular genetic markers of SCD are being actively studied, which can be used in developing a strategy for diagnosing predisposition and preventing SCD in individuals with both known and unknown cardiovascular diseases. The use of the familial approach significantly increases the percentage of successful molecular autopsy. For example, in the Spanish familial study it was 80,4%, while among the deceased probands only 23,3% [11]. Of course, next-generation sequencing (NGS) (whole exome sequencing) in SCD is not a panacea, but a step in the right direction [12]. There are still many difficulties to overcome in accumulating information and experience in interpreting NGS results. The result of whole exome sequencing of 17 super-long-lived people (110 years and older) is very significant in this sense. At the time of the work, there were <100 such people in the world. There was a very interesting finding: in the genome of one of the centenarians, a pathogenic mutation in the *DSC2* gene was found, predisposing to right ventricular arrhythmogenic cardiomyopathy (CM) [13].

Several studies have found variants of the copy number variation (CNV) responsible for cardiovascular diseases associated with SCD, but very little work has been done on large groups of patients and mostly focus on a specific SCD-related disease. Study by Mates J, et al. (2018) presented the results of search for CNV in SCD-associated genes in a large group of patients (n=1765). Patients suffered sudden unexplained death or had a hereditary heart disease (CM or channelopathy). Thirty-six CNVs (2%) were identified, most of which appear to play a pathogenic role. The frequency of CNVs among cases of sudden unexplained death in patients with CM or channelopathy was 1,4% (8/587), 2,3% (20/874) and 2.6% (8/304), respectively. The detection rate was particularly high in arrhythmogenic CM (5,1%), long QT syndrome (LQTS) (4,7%), and dilated CM (4,4%). The authors believe that CNV analysis should be performed as part of routine genetic testing of SCD cases and in patients with SCD-associated diseases [14].

Heterozygous mutations in the *SCN5A* gene are associated with various arrhythmia phenotypes. The severity of the phenotype can range from electrocardiographic abnormalities (mild phenotype) to symptomatic arrhythmias leading to syncope, cardiac arrest, and SCD (severe phenotype), even among members of the same family with the same mutation. Risk stratification for carriers of *SCN5A* gene mutations remains an unsolved problem. In a large pedigree with a heterozygous *SCN5A* gene mutation with loss of function (1936delC, Q646RfsX5), 22 carriers of the mutation were found. Analysis of the *SCN5A* gene promoter region (2800 BP) identified 2 single-nucleotide polymorphisms associated with the severity of the disease. That is, the presence of specific promoter variants increases the risk of severe phenotype in heterozygous carriers of the *SCN5A* mutation with loss of function. The authors failed to detect the supposed differences in the methylation of genes associated with *SCN5A* [15].

In Denmark, a panel of 100 genes was sequenced in 72 cases of SCD under the age of 50. In 52 cases, the cause could not be determined during autopsy. In 15 (28,9%) cases, probable causal mutations were found. Although interpretation of NGS data is a complex task, it helps first the forensic expert in determining the true cause of death, and then allows the cardiologist to help relatives [16].

In a large international study, 302 cases of SCD were analyzed, including 82 surviving probands with families (mean age, 24 years; men, 65%), and a panel of 77 genes was sequenced [17]. A pathogenic

Table 1

Variants probably associated with SCD

Patient	Gene	Single-nucleotide polymorphism	Amino acid replacement	PolyPhen	Mutation Taster	FATHMM	PROVEAN	LIST (score)	gnomAD, MAF	ClinVar
2	*KCNA5*	rs139614200	p.Asp322His	P	D	D	D	0,877	0,00009239	CI
2	*TGFB3*	New	p.Asp109Val	D	D	T	D	0,847	-	-
12	*NEB*	New	Tyr5878Ser	D	D	T	D	0,741	-	-
12	*PDX1*	New	Met36Arg	P	D	D	N	0,714	-	-
18	*FLNC*	rs201572079	p.Gly553Ser	D	D	D	D	0,805	0,0002705	CI, D
19	*FLNC*	rs199935488	p.Thr435Met	P	N	D	D	0,918	0,00004834	U
22	*PLEC*	New	p.Ile2550Asn	D	D	T	D	0,901	-	-
30	*APOB*	New	p.Glu2008Asn	D	D	T	D	0,792	-	-
31	*MYH2*	rs762121316	p.Arg783Ter	-	-	D	-	-	0,00009556	D
34	*KCND3*	rs35027371	p.Arg549His	D	N	D	D	0,920	0,00009900	U
34	*KCNH2*	rs143512106	p.Arg885Cys	P	D	D	D	0,905	0,0002	U
34	*SNTA1*	rs770192754	p.Glu278Lys	N	D	-	D	0,876	0,00001	-
35	*AKAP9*	rs61757671	p.Glu2025Lys	P	D	T	D	0,824	0,001121	CI, P

Abbreviations: D — damaging/deleterious, P — probable damage, T — tolerated, N — polymorphism, MAF — minor allele frequency, U — uncertain significance, CI — conflicting interpretations.

or probable pathogenic variant was detected in 40 out of 302 cases of SCD (13%). The combination of molecular autopsy with clinical assessment in the families of surviving patients increased the diagnostic yield to 39% [17].

Thus, despite the research conducted in the field of molecular autopsy in SCD, there are still many questions that require further study.

Material and methods

The study included autopsy material of 37 suddenly deceased male patients with a diagnosis of SCD aged 20 to 45 years. The average age of men included in the SCD group was 32,4±6,4 years. The study was approved by the Ethics Committee of Research Institute of Therapy and Preventive Medicine.

The diagnosis was made using the SCD criteria of the World Health Organization and the European Society of Cardiology [18]. Taking into account the limited information about the time of death, the SCD group includes deaths that developed within one hour or within no more than 24 hours without witnesses of death and were considered according to forensic research as a cardiac death. Exclusion criteria were morphological changes in cardiac tissues characteristic of myocardial infarction and CM. In addition, the group excluded persons with alcohol or drug intoxication, which could cause or contribute to fatal outcome against the background of cardiovascular disease.

During the forensic analysis, the myocardial tissue weighing 5-10 g was taken from the deceased, which was further stored at a temperature of -20° C in a freezer until the DNA extraction. Genomic DNA was isolated by a modified phenol-chloroform method from the myocardium of men who died from SCD. The quality of the analyzed DNA was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., USA). Whole exome sequencing was performed on the HiSeq 1500 system (Illumina, USA). The SureSelect Focused Exome kit (Agilent Technologies, Inc., USA) was used to prepare the libraries. Bioinformatic analysis of sequencing data was performed using the Genomenal NGSWizard software [19]. The obtained variants were annotated using the genome database (gnomAD) [20] and ClinVar [21]. The analysis also took into account the data of *in silico* testing using the PolyPhen-2, Mutation Taster, SIFT, PROVEAN, FATHMM, LIST programs [22-26]. The variants were selected on the basis of the following criteria: localization of the variant (missense or nonsense mutations), replacement of splicing sites with a rare allele frequency <1%, absence of homozygotes (according to gnomAD data). Variants were selected, the pathogenicity of which was predicted in at least four cases out of five (for LIST, based on the predictor coefficient >0,7). Variants with uncertain significance and conflicting interpretations of pathogenicity according to the ClinVar database were also included in the analysis. Variants with a frequency of >0,01% and synonymous variants were excluded from the analysis for autosomal dominant diseases [27]. The search for functionally significant substitutions was carried out, first of all, in the genes associated with LQTS

Table 2

Variability of the percentage of found mutations in SCD in studies

Number of SCD	Number of genes	%	Familial analysis	Age	Auhor, year	Note
10	174	30 — P 50 — U	+	19-40	Hellenthal N, 2017	
27	95	44,4	-	-	Chanavat V, 2016	
28	exome	43 — total 21 — P, PP	+	18,4±7,8	Shanks GW, 2017	
32	100	44	-	1-19	Anderson JH, 2016	SCD in physical activity
34	exome	29,4	-	33,07±12,85 m 23,62±15,34 f	Neubauer J, 2018	
42	242	23	+	30,2±16,1	Jiménez-Jáimez J, 2017	SCD, survivors
44	80	27,3	-	30,7±7,4	Zhang L, 2016	
52	100	28,9	-	up to 50	Hertz CL, 2016	
61	100	34	-	1-50	Christiansen SL, 2016	
72	35	29	+	5-40	Mak CM, 2019	
119	55	30 — PP 10 — P	-	up to 50	Sanchez O, 2016	
197	6	5	-	22,6±14,4	Raju H, 2019	
302	77	13 — P, PP	+	24 (17-33)	Lahrouchi N, 2017	SCD, survivors
600	49	2,5 — P, PP	-	72±9	Khera AV, 2019	Prospective

Abbreviations: PP — probably pathogenic substitution, SCD — sudden cardiac death, P — pathogenic substitution, U — uncertain meaning.

according to GeneReviews data [28] — *KCNQ1, KCNH2, SCN5A, AKAP9, ANK2, CACNA1C, CALM1, CALM2, CAV3, KCNE1, KCNE2, KCNJ5 SCN4B, SNTA1*, as well as *MYH2, APOB, KCNA5, TGFB3, NEB, PDX1, FLNC, PLEC, KCND3*. The choice of genes for analysis remains an unsolved problem: at the first phase of research, the number of LQTS-associated genes increased year after year until it reached 17. However, at the beginning of 2020, studies were published in which the contribution of all these genes in to LQTS was questioned [29, 30].

Results and discussion

Of 37 samples with SCD, 13 probably pathogenic missense mutations were found in 9 samples (24,3%). Of the 13 probably pathogenic variants, 5 were new. The results are presented in Table 1.

KCNH2 gene, OMIM 152427 (potassium channel, voltage-gated, subfamily H, member 2), cytogenetic location 7q36.1. Mutations in this gene can lead to the development of two syndromes — LQTS 2 and short QT syndrome 1 [31]. The dbSNP database contains information about 10,120 changes in the nucleotide sequence in this gene, of which 281 are classified as pathogenic, 139 — as probably pathogenic, 340 — with indeterminate significance, and 25 more for which the researchers did not agree on the category [32]. According to the HuGE Navigator database, most publications are devoted to the study of the relationship of this gene with different types of arrhythmias and related phenotypes — SCD, atrial fibrillation, syncope, Brugada syndrome, etc. [33]. In one of the studied samples, the substitution c.2653C>T was found, which leads to the replacement of the Arg885Cys amino acid in the protein (rs143512106); the frequency of rare allele is 0,0002. We could not find any references to this substitution in literature, however, according to the programs for predicting the substitution pathogenicity, it is categorized as probably pathogenic. In the same sample, another new substitution was found in the *SNTA1* gene: c.832G>A, which leads to the substitution of the amino acid Glu278Lys in the protein (rs770192754). *SNTA1* gene, OMIM 601017 (syntrophin, alpha-1), cytogenetic location 20q11.21. Mutations in this gene can lead to the development of LQTS 12 [31]. The dbSNP database contains information on 8684 changes in the nucleotide sequence in this gene [32]. When tested with impact assessment programs, the Glu278Lys substitution was categorized as a conflict of interpretation. Whether the carriage of two rare variants at the same time (*KCNH2*, Arg885Cys, and *SNTA1*, Glu278Lys) somehow influences the risk of developing SCD is unambiguous. There are no reliable

generally accepted algorithms for assessing the effect of the carriage of several mutations in different genes on the phenotype, but the very concept of oligogenic diseases has already become commonplace, and information about these diseases is being accumulated [34, 35]. Thus, for the combination of the R800L mutation in the *SCN5A* gene with the A261V mutation in the *SNTA1* gene, an increase in the clinical manifestations of LQTS was shown. In addition, the authors on cell culture proved that carriers of two mutations have an increase in the function of channels with *SCN5A*, which increases the duration of the action potential and can lead to the LQTS phenotype [36]. The A261V substitution is located in the same exon of the gene as the Glu278Lys we discovered. In addition to intergenic interactions, in this pilot analysis we could not take into account and check the modifying effect of polymorphisms on the penetrance of mutations, although there are indications in the literature [15], including in relation to the *SNTA1* gene: the P74L polymorphism significantly affects the registered currents in the presence of A257G mutation [37]. These facts confirm the growing complexity of genetic risk stratification of arrhythmia and SCD.

Of 37 samples with SCD, probably pathogenic missense mutations were found in 24,3% of samples. Is it a lot or a little? Table 2 shows the results of 14 SCD studies. They show how difficult it is to compare the data obtained. On average, in 30% of the samples, the researchers were able to find pathogenic or probably pathogenic variants. But upon a more detailed comparison of the results, they turn out to be very heterogeneous in terms of:

1) Number of patients.
2) Men-to-women ratio.
3) Age: minimum, mean, maximum.
4) Number of analyzed genes.
5) Composition of the studied groups: only deceased SCD; deceased SCD and survivors, only probands with SCD; probands and their relatives.
6) Inclusion of family history and examination data of living relatives and without this data.
7) With known death circumstances and health conditions prior to SCD and without this data.
8) Case control studies; prospective cohort studies.
9) Inclusion/exclusion criteria, SCD against the background of only channelopathy and CM, the coronary artery disease and aortopathies (including aortic dissection) [3], against the background of familial hypercholesterolemia [38].
10) Race and ethnicity of the deceased SCD.
11) Proportion of mutations in genes associated with phenotypes of increased SCD risk. In some cases, mutations in the genes of channelopathies predominate, in others — CM, etc.

Therefore, each study on SCD using NGS methods presents unique data, which must be compared very carefully, taking into account the above differences. There remain the difficulties of analyzing representatives of the Russian population associated with their underrepresentation in large projects [39].

Another problem that reduces the efficiency of the search for causal mutations is the skipping of large segments of DNA during exome sequencing. Gotway G, et al. studied and compared the results of sequencing 36 exomes in 3 clinical laboratories (2012-2016). For genes of the consensus coding sequence, the average number of fully covered genes varied significantly: 12184 (69%), 11687 (66%), and 5989 (34%) for laboratories A, B, and C, respectively. That is, there is a significant inconsistency in the coverage of exome genes between laboratories [40]. There are still no convenient tools for answering such a seemingly simple question: in the analyzed DNA, no known pathogenic variants were found in hundreds of genes because they are not there, or because they have not been analyzed with acceptable quality? The quality of available databases, programs used, algorithms and criteria for assessing the pathogenicity of the found variants is improving. However, it is still far from achieving a good reproducible result. The scale of interpretation problem can be illustrated by the example of the results obtained during the Trans-Omics for Precision Medicine (TOPMed) program, which is aimed at elucidating the genetic architecture and biology of diseases of the heart, lungs, blood, sleep disorders, with the ultimate goal of improving diagnosis, treatment and prevention. In 53,581 samples, more than 400 million single nucleotide and insertion-deletion variants were found, and 97% of them have a frequency of <1% and 46% are singletons. On the one hand, this is a big step towards a significant expansion of opportunities for studying the contribution of rare and non-coding sequence variants to phenotypic manifestations [41]. On the other hand, this shows what a colossal amount of information has yet to be figured out. In the meantime, the most accessible approach is phenotypic and genetic analysis of healthy and sick relatives of the proband, which, as shown by many researchers, significantly increases the efficiency of the search for causal mutations. Although, of course, this approach does not solve all problems, but requires a significant investment of time and labour Zaragoza MV, et al. (2016) attempted to understand the underlying genetic mechanisms that cause sick sinus syndrome and to identify potential modifiers that could lead to intrafamilial variation in a multi-generational family. Sixty-three-year-old male proband with a family

history (>10) of sinus node dysfunction, ventricular arrhythmia, CM, heart failure, and sudden death. They successfully sequenced 94% of the proband's exome, found 128,563 unique variants, of which 108795 (85%) were in 16,319 genes out of 19,056 target genes. To identify possible mutations, the authors focused on 2,000 variants located in 237 genes out of 283 known genes for arrhythmias, channelopathies, and CM. After filtration of them taking into account zygosity, influence on protein, information in databases, 41 variants remained in 33. Ultimately, they selected 9 confirmed variants with allele frequencies <1% for family analysis and found a new substitution c.357-2A>G at the splice site in the *LMNA* gene, as well as a number of rare or new variants in the *HCN4, MYBPC3, PKP4, TMPO, TTN, DMPK* and *KCNJ10* genes as potential modifiers [42].

The 2017 Russian Clinical Guidelines on SCD suggest to consider conducting a postmortem genetic testing in case of suspected congenital structural heart disease or congenital arrhytmia/conduction disorder as a possible cause of SCD [5]. The practical implementation of these recommendations requires the cooperation of experienced specialists in the field of forensic medicine, laboratory genetics, and cardiology.

Conclusion

This pilot study provides following conclusions: it is necessary to continue molecular autopsy research in Russia, to increase the effectiveness of detecting causal mutations, to reduce the age of patients with SCD included in the study, to study the families of deceased.

Relationships and Activities. This paper was supported by the Russian Foundation for Basic Research grant № 17-29-06026, partially by the grant № NSh-2595.2020.7, and budget projects № 0324-2016-0002, 0120.0502961

References

1. Morini E, Sangiuolo F, Caporossi D, et al. Application of Next Generation Sequencing for personalized medicine for sudden cardiac death. Front Genet. 2015;6:55.
2. Mizusawa Y. Recent advances in genetic testing and counseling for inherited arrhythmias. J Arrhythm. 2016;32(5):389-97.
3. Khera AV, Mason-Suares H, Brockman D, et al. Rare Genetic Variants Associated With Sudden Cardiac Death in Adults. J Am Coll Cardiol. 2019;74(21):2623-34. doi:10.1016/j.jacc.2019.08.1060.
4. Shljahto EV, Arutjunov GP, Belenkov YuN, Ardashev AV. Recommendations for risk assessment and prevention of sudden cardiac death. Archive of internal medicine. 2013;(4):5-15. (In Russ.)
5. Revishvili ASh, Neminuschiy NM, Batalov RE, et al. All-Russian clinical guidelines for controlling the risk of sudden cardiac arrest and sudden cardiac death, prevention and first aid. Vestnik aritmologii. 2017;(89):2-104. (In Russ.)
6. Faragli A, Underwood K, Priori SG, Mazzanti A. Is There a Role for Genetics in the Prevention of Sudden Cardiac Death? J Cardiovasc Electrophysiol. 2016;27(9):1124-32. doi:10.1111/jce.13028.
7. Neubauer J, Lecca MR, Russo G, et al. Exome analysis in 34 sudden unexplained death (SUD) victims mainly identified variants in channelopathy-associated genes. Int J Legal Med. 2018;132(4):1057-65. doi:10.1007/s00414-018-1775-y.
8. Loporcaro CG, Tester DJ, Maleszewski JJ, et al. Confirmation of cause and manner of death via a comprehensive cardiac autopsy including whole exome next-generation sequencing. Arch Pathol Lab Med. 2014;138(8):1083-9. doi:10.5858/arpa.2013-0479-SA.
9. Hertz CL, Christiansen SL, Ferrero-Miliani L, et al. Next-generation sequencing of 34 genes in sudden unexplained death victims in forensics and in patients with channelopathic cardiac diseases. Int J Legal Med. 2015;129(4):793-800. doi:10.1007/s00414-014-1105-y.
10. Narula N, Tester DJ, Paulmichl A, et al. Post-mortem Whole exome sequencing with gene-specific analysis for autopsy-negative sudden unexplained death in the young: a case series. Pediatr Cardiol. 2015;36(4):768-78. doi:10.1007/s00246-014-1082-4.
11. Jiménez-Jáimez J, Alcalde Martínez V, Jiménez Fernández M, et al. Clinical and Genetic Diagnosis of Nonischemic Sudden Cardiac Death. Rev Esp Cardiol (Engl Ed). 2017;70(10):808-16. doi:10.1016/j.rec.2017.04.024.
12. Beauséjour Ladouceur V, Abrams DJ. Whole-Exome Molecular Autopsy After Exertional Sudden Cardiac Death: Not a Panacea but a Step in the Right Direction. Circ Cardiovasc Genet. 2016;9(3):210-2. doi:10.1161/CIRCGENETICS.116.001484.
13. Gierman HJ, Fortney K, Roach JC, et al. Whole-genome sequencing of the world's oldest people. PLoS One. 2014;9(11):e112430. doi:10.1371/journal.pone.0112430.
14. Mates J, Mademont-Soler I, Del Olmo B, et al. Role of copy number variants in sudden cardiac death and related diseases: genetic analysis and translation into clinical practice. Eur J Hum Genet. 2018;26(7):1014-25. doi:10.1038/s41431-018-0119-1.
15. Park JK, Martin LJ, Zhang X, et al. Genetic variants in SCN5A promoter are associated with arrhythmia phenotype severity in patients with heterozygous loss-of-function mutation. Heart Rhythm. 2012;9(7):1090-6. doi:10.1016/j.hrthm.2012.02.023.
16. Hertz CL, Christiansen SL, Ferrero-Miliani L, et al. Next-generation sequencing of 100 candidate genes in young victims of suspected sudden cardiac death with structural abnormalities of the heart. Int J Legal Med. 2016;130(1):91-102. doi:10.1007/s00414-015-1261-8.
17. Lahrouchi N, Raju H, Lodder EM, et al. Utility of Post-Mortem Genetic Testing in Cases of Sudden Arrhythmic Death Syndrome. J Am Coll Cardiol. 2017;69(17):2134-45. doi:10.1016/j.jacc.2017.02.046.
18. Priori SG, Aliot E, Blømstrom-Lundqvist C, et al. The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology. Europace. 2015;17(11):1601-87.
19. NGSWizard на платформе Genomenal https://ru.genomenal.com/.
20. GNOMAD https://gnomad.broadinstitute.org/.
21. ClinVar https://www.ncbi.nlm.nih.gov/clinvar/.
22. Mutation Taster http://www.mutationtaster.org/.
23. PolyPhen-2 http://genetics.bwh.harvard.edu/pph2/.
24. PROVEAN http://provean.jcvi.org/index.php.
25. FATHMM http://fathmm.biocompute.org.uk/.
26. LIST http://list.msl.ubc.ca/.
27. Ryzhkova OP, Kardymon OL, Prohorchuk EB, et al. Guidelines for the interpretation of massive parallel sequencing variants (update 2018, v2). Medical genetics. 2019;18(2):3-23. (In Russ.) doi:10.25557/2073-7998.2019.02.3-23.
28. GeneReviews https://www.ncbi.nlm.nih.gov/books/NBK1129/.
29. Adler A, Novelli V, Amin AS, et al. An International, Multicentered, Evidence-Based Reappraisal of Genes Reported to Cause Congenital Long QT Syndrome. Circulation. 2020;141(6):418-28. doi:10.1161/CIRCULATIONAHA.119.043132.
30. Waddell-Smith KE, Skinner JR, Bos JM. Pre-Test Probability and Genes and Variants of Uncertain Significance in Familial Long QT Syndrome. Heart Lung Circ. 2020;29(4):512-9. doi:10.1016/j.hlc.2019.12.011.
31. OMIM Online Mendelian Inheritance in Man http://omim.org/.
32. dbSNP https://www.ncbi.nlm.nih.gov/snp/.
33. HuGE Navigator https://phgkb.cdc.gov/PHGKB/hNHome.action.
34. Shigemizu D, Aiba T, Nakagawa H, et al. Exome Analyses of Long QT Syndrome Reveal Candidate Pathogenic Mutations in Calmodulin-Interacting Genes. PLoS ONE. 2015;10(7):e0130329. doi:10.1371/journal.pone.0130329.
35. Human genetics according to Vogel and Motulski. Problems and approaches. Ed. Speicher MR, Antonarakis SE, Motulsky AG. St. Petersburg: Publishing N-L, 2013. p.1056. (In Russ.)
36. Hu RM, Tan BH, Orland KM, et al. Digenic inheritance novel mutations in SCN5a and SNTA1 increase late I(Na) contributing to LQT syndrome. Am J Physiol Heart Circ Physiol. 2013;304(7):H994-H1001. doi:10.1152/ajpheart.00705.2012.
37. Cheng J, Norstrand DW, Medeiros-Domingo A, et al. LQTS-associated mutation A257G in α1-syntrophin interacts with the intragenic variant P74L to modify its biophysical phenotype. Cardiogenetics. 2011;1(1). pii:136.
38. Larsen MK, Nissen PH, Kristensen IB, et al. Sudden cardiac death in young adults: environmental risk factors and genetic aspects of premature atherosclerosis. J Forensic Sci. 2012;57(3):658-62. doi:10.1111/j.1556-4029.2011.02028.x.
39. Barbitoff YA, Skitchenko RK, Poleshchuk OI, et al. Whole-exome sequencing provides insights into monogenic disease prevalence in Northwest Russia. Mol Genet Genomic Med. 2019;7(11):e964. doi:10.1002/mgg3.964.
40. Gotway G, Crossley E, Kozlitina J, et al. Clinical Exome Studies Have Inconsistent Coverage. Clinical Chemistry. 2020;66(1):199-206. doi:10.1093/clinchem.2019.306795.
41. Taliun D, Harris DN, Kessler MD, et al. Sequencing of 53,831 diverse genomes from the NHLBI TOPMed Program. BioRxiv. 2019. 563866. doi:10.1101/563866.
42. Zaragoza MV, Fung L, Jensen E, et al. Exome Sequencing Identifies a Novel LMNA Splice-Site Mutation and Multigenic Heterozygosity of Potential Modifiers in a Family with Sick Sinus Syndrome, Dilated Cardiomyopathy, and Sudden Cardiac Death. PLoS One. 2016;11(5):e0155421. doi:10.1371/journal.pone.0155421.

https://russjcardiol.elpub.ru
doi:10.15829/1560-4071-2020-4040

ISSN 1560-4071 (print)
ISSN 2618-7620 (online)

Deoxyribonucleic acid methylation in the enhancer region of the *CDKN2A/2B* and *CDKN2B-AS1* genes in blood vessels and cells in patients with carotid atherosclerosis

Koroleva Yu. A.[1], Markov A. V.[1], Goncharova I. A.[1], Sleptsov A. A.[1], Babushkina N. P.[1], Valiakhmetov N. R.[2], Sharysh D. V.[1], Zarubin A. A.[1], Kuznetsov M. S.[2], Kozlov B. N.[2], Nazarenko M. S.[1]

Aim. Comparative analysis of the deoxyribonucleic acid (DNA) methylation level in the enhancer region of the *CDKN2A/2B* and *CDKN2B-AS1* genes (9p21.3 locus) in vessels with/without atherosclerotic lesions, as well as in leukocytes of patients with clinically relevant carotid artery (CA) atherosclerosis and healthy individuals.

Material and methods. The group of patients with clinically relevant atherosclerosis included 22 individuals with severe stenosis (>80%) of CA. Samples of atherosclerotic plaques, presenting CA regions, and great saphenous veins, as well as peripheral blood samples (leukocytes) were obtained from patients. The control group consisted of 14 individuals with the mild CA stenosis (≤24%) and without hemodynamically relevant changes; peripheral blood samples were obtained from each of them. DNA methylation level was assessed by targeted bisulfite sequencing of amplicons.

Results. The tissue-specific methylation of 31 CpG-site in the *CDKN2A/2B* and *CDKN2B-AS1* gene enhancer was established: the vascular tissues significantly differed from the peripheral blood leukocytes. At the same time, there was an increase in the methylation level of both certain CpG sites and whole analyzed CA region affected by atherosclerosis (48,6 [34,8; 62,0]%), compared with intact vessels, both arteries (25,2 [23,1; 41,60]%, p=0,0001) and veins (35,0 [31,6; 40,0]%, p=0,0039). Patients had lower methylation levels in all CpG sites in blood leukocytes compared to blood vessel samples (8,7 [6,1; 9,7]%; p<0,05). At the same time, the level of DNA methylation in the blood leukocytes of atherosclerotic patients does not differ from that in healthy individuals (9,3 [8,3; 13,6]%; p>0,8).

Conclusion. In the present study, the relationship between an increase in the DNA methylation in the enhancer of the *CDKN2A/2B* and *CDKN2B-AS1* genes in CA and their atherosclerotic lesions was revealed, as well as the tissue-specific DNA methylation between vessels and peripheral blood leukocytes.

Key words: atherosclerosis, DNA methylation, 9p21.3, *CDKN2A/2B, CDKN2B-AS1*.

Relationships and Activities: none.

[1]Research Institute of Medical Genetics, Tomsk National Research Medical Center, Tomsk; [2]Cardiology Research Institute, Tomsk National Research Medical Center, Tomsk, Russia.

Koroleva Yu. A.* ORCID: 0000-0003-1498-6934, Markov A. V. ORCID: 0000-0002-5824-6439, Goncharova I. A. ORCID: 0000-0002-9527-7015, Sleptsov A. A. ORCID: 0000-0003-3226-1750, Babushkina N. P. ORCID: 0000-0001-6133-8986, Valiakhmetov N. R. ORCID: 0000-0001-7969-7020, Sharysh D. V. ORCID: 0000-0003-2173-2772, Zarubin A. A. ORCID: 0000-0001-6568-6339, Kuznetsov M. S. ORCID: 0000-0002-1975-043X, Kozlov B. N. ORCID: 0000-0002-0217-7737, Nazarenko M. S. ORCID: 0000-0002-0673-4094.

*Corresponding author:
yuliya.koroleva@medgenetics.ru

Received: 13.08.2020
Revision Received: 31.08.2020
Accepted: 07.09.2020

For citation: Koroleva Yu. A., Markov A. V., Goncharova I. A., Sleptsov A. A., Babushkina N. P., Valiakhmetov N. R., Sharysh D. V., Zarubin A. A., Kuznetsov M. S., Kozlov B. N., Nazarenko M. S. *Russian Journal of Cardiology*. 2020;25(10):4060. (In Russ.) doi:10.15829/1560-4071-2020-4060

Single nucleotide polymorphisms (SNPs) at the 9p21.3 locus are associated with cardiovascular disease continuum (hypertension, atherosclerosis of coronary and carotid arteries (CA), myocardial infarction, ischemic stroke, dyslipidemia, obesity, type 2 diabetes), as well as significant parameters (blood pressure level, body mass index, blood glucose level) [1].

Most SNPs of this locus are linked to each other in an extended haplotype block (~53 kb), which encompasses a cluster of genes for cyclin-dependent kinase inhibitor: *CDKN2A* (encodes p14/ARF and p16/INK4A) and *CDKN2B* (encodes p15/INK4B). The products of these genes are involved in the regulation of the cell cycle and proliferation [2, 3]. Both genes, *CDKN2A* and *CDKN2B*, are read from deoxyribonucleic acid (DNA) in the reverse direction, while the *CDKN2B-AS1* gene is transcribed in the forward direction, the product of which is the noncoding RNA in the INK4 locus [3].

It has been shown that ANRIL is a component of many gene pathways involved in cell proliferation, adhesion, senescence, and apoptosis — the key mechanisms of atherosclerotic arterial lesions [4, 5]. There is evidence that SNPs at the 9p21.3 locus cause an increase in the expression of ANRIL in vascular cells, which suppresses *CDKN2A/2B*, which, in turn, enhances cell proliferation and promotes the development of atherosclerosis [2]. The association of polymorphisms of the 9p21.3 locus with atherosclerosis is also explained by the presence in this region of long-range enhancers altering the functional activity of genes of this locus, including CDKN2A/2B. However, the detailed mechanism of the relationship between genetic variants and the pathological phenotype remains unknown [3].

At the same time, the development of atherosclerosis as a multifactorial disease can be caused not only by genetic variants, but also by epigenetic modifications that regulate gene expression without disrupting the primary DNA nucleotide sequence [6]. One of the most studied epigenetic mechanisms of regulation of gene function is the methylation of CpG dinucleotides, the so-called CpG islands. Basically, the study of DNA methylation is carried out in relation to gene promoters, and methylation of CpG islands in their promoter regions correlates with the silencing of gene transcription. However, there is evidence that hypomethylation of enhancer DNA, allowing the binding of transcription factors, also leads to the activation of transcription of corresponding genes [7].

There are low number of studies devoted to the analysis of DNA methylation of the 9p21.3 locus in blood vessels and cells in cardiovascular disease continuum [8-10]. In these studies, the level of DNA methylation in the region of CpG islands of the *CDKN2A/2B* gene promoters is analyzed in only one tissue in the vascular wall, or peripheral blood leukocytes in patients with atherosclerosis, including those complicated by acute vascular events.

The aim of this study was a comparative analysis of DNA methylation in the enhancer region of *CDKN2A/2B* and *CDKN2B-AS1* genes in the tissues of vascular wall, as well as in the leukocytes of patients with clinically relevant CA atherosclerosis and relatively healthy individuals.

Material and methods

Creation of samples and examination of patients with clinically relevant CA atherosclerosis was carried out on the basis of the Cardiology Research Institute (Tomsk National Research Medical Center). The general inclusion criteria of individuals in the study were the absence of family ties between individuals, Caucasian race, and the absence of cancer. The study was approved by the ethics committee of the Research Institute of Medical Genetics (Tomsk National Research Medical Center). All participants signed informed consent.

The group of patients with clinically relevant atherosclerosis consisted of 22 people aged 53-77 years (men, 17; women, 5) (Table 1). In all patients, ultrasound revealed severe CA stenosis (>80%), which is an indication for carotid endarterectomy. Prior to surgery, peripheral blood samples (leukocytes) were taken from each patient.

By surgery, samples of atherosclerotic plaques (ASP), macroscopically unchanged presenting areas of CA and great saphenous veins were obtained from all patients. Each vessel sample was examined and cleaned from calcification masses, lipid deposits and blood clots, washed in sterile saline in order to remove blood clots. Tissue samples were frozen in liquid nitrogen and stored at -80° C until the examination.

Histological examination of 20 ASP samples with hematoxylin-eosin staining was carried out (Table 1). Diapedesis of erythrocytes in the arterial wall was observed in 15% of cases, as well as the pronounced mononuclear infiltration, cap defect — in 10%, calcification — in 50%. Most (59,1%) of the analyzed ASP samples were classified as type V (fibroatheroma) [18]. The remaining 7 samples were classified as type VI due to signs of ASP destabilization (surface defects, hemorrhage, mononuclear cells in ASP).

The control group consisted of 14 individuals without cardiovascular disease —10 men and 4 women aged 53 to 78 years (Table 1). All individuals underwent an ultrasound, which revealed the initial stages of CA atherosclerosis (⩽24%) without hemo-

Table 1

Clinical characteristics of the groups included in the study

Parameter	Patients with clinically relevant CA atherosclerosis (N=22)	Control group of healthy individuals (N=14)
Clinical parameters		
Sex (men: women)	17:5	10:4
Age, years (Q2 [Q1; Q3])	64 [60; 69]	66 [59; 71]
BMI kg/m^2 (Q2 [Q1; Q3])	27 [26; 31]	29 [26; 32]
History of myocardial infarction, abs. (%)	14 (63,6)	0 (0,0)
History of coronary artery disease, abs. (%)	17 (77,3)	0 (0,0)
History of TIA, stroke, abs. (%)	4 (18,2)	0 (0,0)
Hypertension, abs. (%)	21 (95,5)	8 (56,1)
Smoking, abs. (%)	17 (77,3)	4 (28,6)
Diabetes, abs. (%)	6 (27,3)	2 (14,2)
CA ultrasound (Q2 [Q1; Q3])		
Stenosis degree, %	80 [75; 81]	24 [19; 26]
Histological type of ASP (AHA classification, 1995) [18]		
Type V: fibroateroma with possible calcification	13 (59,1%)	–
Type VI: mixed ASP with possible surface defects, hemorrhage or blood clots	7 (31,8%)	–
Not classified	2 (9,1%)	–
Laboratory data (Q2 [Q1; Q3])		
Glucose, mmol/l	5,5 [5,2; 5,8]	5,6 [5,2; 6,3]
Total cholesterol, mmol/l	4,2 [3,8; 5,2]	5,2 [4,7; 5,3]
Triglycerides, mmol/l	1,5 [1,3; 1,6]	1,3 [1,0; 1,6]
LDL, mmol/l	2,0 [1,8; 2,1]	3,0 [2,8; 3,6]
HDL, mmol/l	1,1 [1,1; 1,4]	1,3 [1,1; 1,7]
Atherogenic index	2,4 [1,8; 2,4]	2,6 [2,3; 3,0]
Medications, abs. (%)		
Anticoagulants/antiplatelet agents	20 (90,8)	2 (14,2)
Antihypertensive agents	16 (72,7)	4 (28,6)
Statins	13 (59,09)	1 (7,1)
Hypoglycemic drugs	5 (22,7)	1 (7,1)

Abbreviations: abs. — number of individuals in the group, ASP — atherosclerotic plaque, CAD — coronary artery disease, BMI — body mass index, HDL — high density lipoproteins, LDL — low density lipoproteins, CVA — cerebrovascular accident, CA — carotid artery, TIA — transient ischemic attack, Q1 — 25% quartile, Q2 — 50% quartile (median), Q3 — 75% quartile.

dynamically related changes. Samples of peripheral blood (leukocytes) were obtained from each individual included in the control group.

The material was DNA isolated from the vascular wall using the QIAamp DNA Mini Kit (Qiagen, USA) and from leukocytes of peripheral blood by the standard phenol-chloroform method. The DNA was then treated with bisulfite using the EZ DNA Methylation Kit (Zymo Research).

To study the DNA methylation level, a region was selected that is localized in exon 2 of *CDKN2B* gene and in intron 1 of *CDKN2B-AS1* gene, which contains the GH09J022005 enhancer of *CDKN2B/2A* and *CDKN2B-AS1* genes, according to the GeneHancer database (Figure 1) [11]. DNA methylation assay was performed by targeted bisulfite sequencing of amplicons using high throughput parallel sequencing.

Primers (F: 5'-TAAAATTAAAAAGTAGTAAGTTATAAGGGG-3' и R: 5'-AACCTACAAACCTATCTAAAACTCACAAAA-3) were used to carry out the polymerase chain reaction of the DNA region containing the enhancer fragment GH09J022005. The selection of primers was performed using the MethPrimer 2 [12]. The studied fragment (chr9:22,005,065-22,005,876, according to genome

Figure 1. Localization and epigenetic context of the GH09J022005 enhancer of *CDKN2A/2B* and *CDKN2B-AS1* genes: the studied region is highlighted in blue. Deciphering of human cell lines studied in the ENCODE project: GM12878 — B-lymphocytes, H1-hESC — embryonic stem cells, K562 — blood cells from a patient with chronic myeloid leukemia, HepG2 — hepatocellular carcinoma cells, HUVEC — umbilical vein endothelial cells.
Note: the color image is available in the electronic version of the issue.

assembly GRCh37/hg19) included 31 CpG sites and SNP — rs3217986, for which an association with severe coronary atherosclerosis and myocardial infarction was previously shown [13, 14].

DNA amplification was carried out on a ProFlex PCR System (ThermoFisher Scientific) according to the program: 95° C — 5 min, 40x (95° C — 30 sec, 62° C — 30 sec, 72° C — 60 sec), 72° C — 5 min. Bisulfite DNA sequencing was performed on a MiSeq system (Illumina). Statistical analysis was performed using R software (version 3.6.2). To assess the methylation levels of CpG sites, nonparametric distribution estimates were used as follows: M [Q1; Q3], where M is the median, Q1 — 1st quartile (25th percentile), Q3 — 3rd quartile (75th percentile). The similarity of the samples in methylation profiles of CpG sites in the analyzed genome region was visualized using the t-distributed Stochastic Neighbor Embedding (t-SNE). Comparison of methylation levels was also carried out using nonparametric criteria. Differences were considered significant at $p<0,05$. Since the methylation levels of all CpG sites were highly correlated (minimal Spearman's Rho value, 0,76), it was inappropriate to use statistical corrections, such as the Bonferroni or Benjamini-Hochberg corrections.

Results and discussion

The analysis of the methylation level of 31 CpG sites in the enhancer region of the *CDKN2A/2B* and *CDKN2B-AS1* genes revealed that the samples of blood vessels (arteries and veins) are clustered separately from the DNA samples of blood leukocytes (Figure 2). Moreover, according to the DNA methylation profile, CA with atherosclerotic lesions are located in the same group with intact blood vessels, and the patients' leukocytes are grouped with those of healthy individuals. Thus, there is a pronounced tissue specificity of methylation of this region for blood vessels and leukocytes, which is consistent with the literature data [15].

An increase in the methylation level of both individual CpG sites and the mean DNA methylation level as a whole in CA and atherosclerotic areas (48,6 [34,8; 62,0]%) in comparison with both arteries (25,2 [23,1; 41,60]%, $p=0,0001$) and veins (35,0 [31,6; 40,0]%, $p=0,0039$; Figures 3A and 3B) was revealed. Differences in DNA methylation level between intact arteries and veins did not reach significance ($p>0,05$). Patients had lower methylation levels in all CpG sites in blood leukocytes compared to blood vessel samples (8,7 [6,1; 9,7]%; $p<0,05$). However, the DNA methylation level in the studied region in leukocytes of patients with atherosclerosis does not differ from those of healthy individuals (9,3 [8,3; 13,6]%; $p>0,8$, Figures 3A and 3B).

The enhancer region of *CDKN2A/2B* and *CDKN2B-AS1* genes contains separate CpG sites (chr9: 22,005,288 and chr9: 22,005,564), which are included in the Illumina microarrays cg19481686 and cg08390209, respectively. Table 2 provides information on the methylation level of these CpG

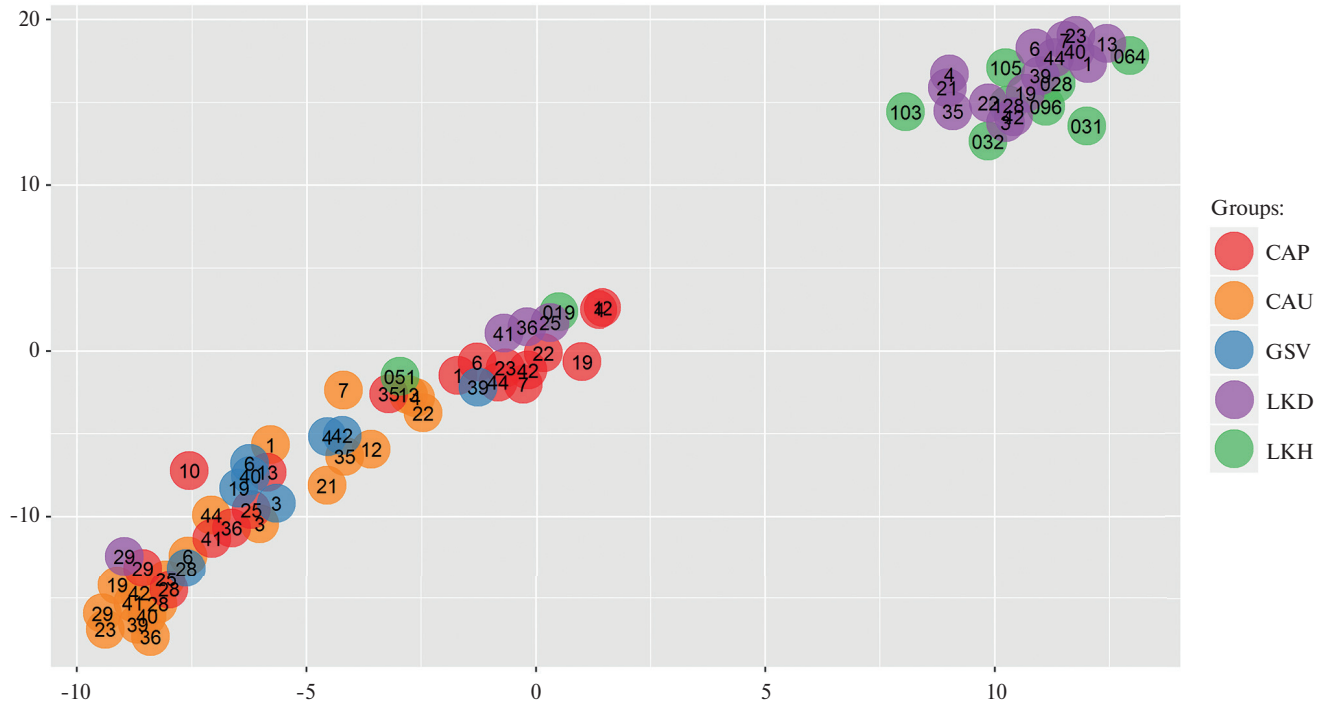


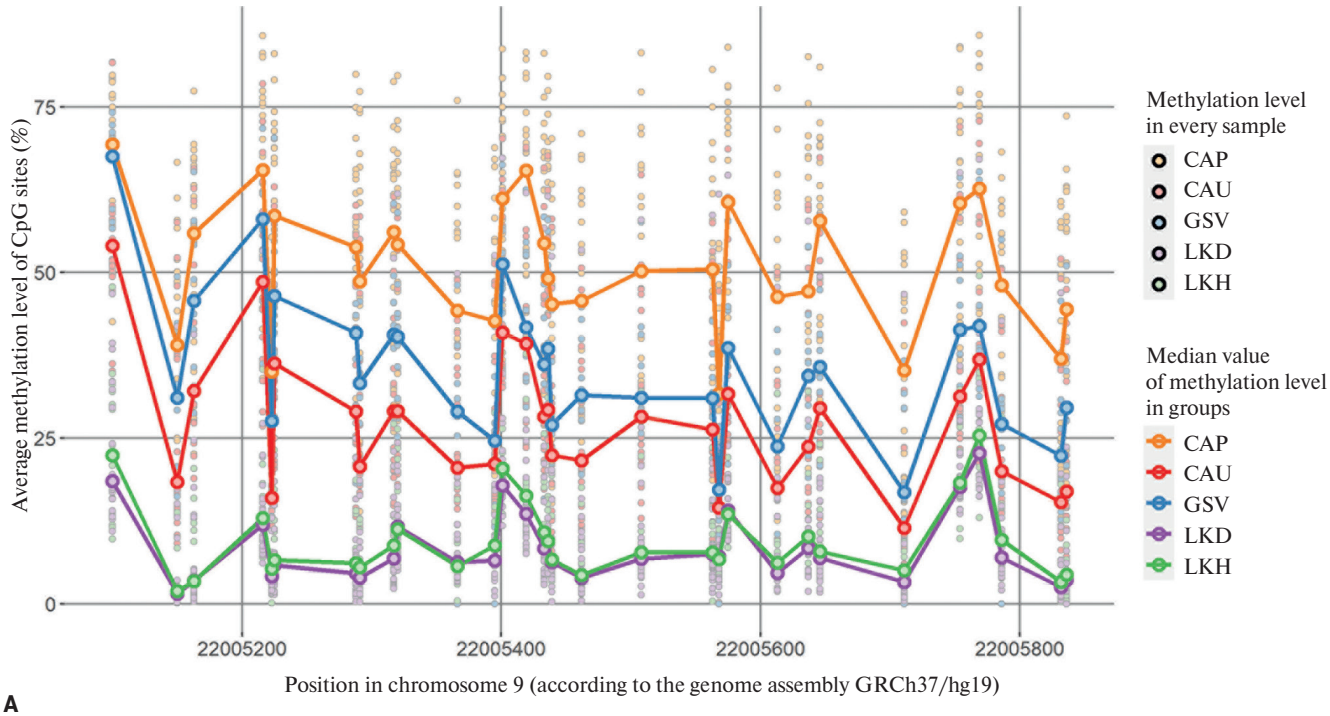
Figure 2. Mutual arrangement and clustering of blood vessels and leukocytes (according to the t-SNE method) depending on the methylation profiles of CpG sites in the enhancer region of *CDKN2A/2B* and *CDKN2B-AS1* genes.
Note: the color image is available in the electronic version of the issue.
Abbreviations: GSV — samples of great saphenous veins, LKD — blood leukocytes of patients with atherosclerosis, LKH — blood leukocytes of the control group, CAP — samples of carotid atherosclerotic plaques, CAU — samples of macroscopically unchanged carotid arteries.

sites according to current study and previously published papers. The methylation level of cg19481686 and cg08390209 in atherosclerotic CA in the present study (53,85 [30,18; 63,57]% and 50,45 [32,24; 60,88]%, respectively) coincides with those in CA obtained from both patients with stroke (55,03 [50,39; 61,24]% and 47,31 [43,50; 56,06]%, respectively) and without a history of stroke (55,27 [49,70; 59,63]% and 50,04 [42,04; 53,88]%, respectively).

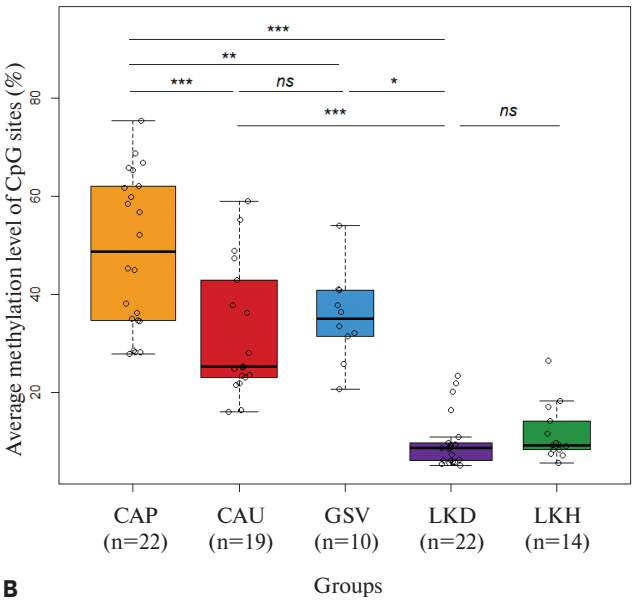
However, in the coronary arteries (69,13 [56,37; 78,95]% and 67,11 [57,87; 72,87]%) and aorta (69,28 [66,95; 70,93]% and 66,52 [60,41; 71,18]%) with atherosclerotic lesions, the methylation level of cg19481686 and cg08390209 is significantly higher than in the CA. At the same time, the methylation level of cg19481686 and cg08390209 in the aorta with atherosclerotic lesions was significantly lower by 9-15% compared to the intact aorta, and the coronary arteries with atherosclerotic lesions had a lower methylation level of these CpG sites by 2-4% than intact internal thoracic arteries, but a higher compared to the great saphenous veins by 4%.

In general, in the vessels of patients with atherosclerosis of various localization, the methylation level of the analyzed CpG sites was significantly higher than that in leukocytes of patients with CA atherosclerosis (4,60 [2,84; 7,76]% and 7,47 [5,44; 8,77]%, respectively), including in stroke (11,38 [9,65; 13,64]% and 12,50 [10,63; 14,23]%, respectively), as well as healthy individuals (6,11 [3,61; 12,25]% and 7,77 [5,92; 11,41]%). At the same time, the methylation level in cg19481686 and cg08390209 in blood leukocytes in patients with atherosclerosis complicated by stroke was significantly 5% higher than in healthy individuals. At the same time, the methylation level of cg19481686 and cg08390209 in blood leukocytes between patients with CA atherosclerosis does not significantly differ from healthy individuals. Thus, in the present study, the relationship between an increase in DNA methylation level of the enhancer of *CDKN2A/2B* and *CDKN2B-AS1* genes in CA and their atherosclerotic lesions was revealed. To resolve the issue of relationship between the DNA methylation level of the analyzed genome region with coronary and aortic atherosclerosis, as well as with the risk of acute vascular events, an additional study is needed with large samples.

It should be noted that the literature contains data on changes in DNA methylation level in 9p21.3 locus in blood vessels and leukocytes, as well as its relationship with atherosclerotic lesions of the arteries. The region of CpG islands of *CDKN2A/2B* and *CDKN2B-AS1* gene promoters is most often analyzed in the studies. In particular, in our previous



A



B

Figure 3. Methylation profiles (**A**) and average methylation level (**B**) of CpG sites in the enhancer of *CDKN2A/2B* and *CDKN2B-AS1* genes in blood vessels and leukocytes.
Notes: ns — not significant, * — p<0,05, ** — p<0,001, *** — p<0,001.
Abbreviations: GSV — samples of great saphenous veins, LKD — blood leukocytes of patients with atherosclerosis, LKH — blood leukocytes of the control group, CAP — samples of carotid atherosclerotic plaques, CAU — samples of macroscopically unchanged carotid arteries.

study, no differences were found in the methylation level of the CpG islands of *CDKN2A/2B* gene promoters in CA ASP and the adjacent macroscopically intact vascular wall in the same patients. However, DNA hypermethylation was noted in the enhancer region of *CDKN2A/2B* and *CDKN2B-AS1* genes, especially in CA ASP [8].

The genome-wide study revealed hypomethylation of the *CDKN2B-AS1* promoter in the chr9: 21,993,116-21,994,101 region and exon 7 of this gene (chr9: 22,056,255-22,056,627), but hypermethylation of exon 8 (chr9: 22,056,256-22,056,628) in the femoral arteries compared with unaffected internal thoracic arteries [16]. At the same time, in a study using bisulfite DNA pyrosequencing, a high methylation level (>55%) of 9 CpG sites (region of *CDKN2B-AS1* promoter, chr9: 21,993,583-21,993,721) in umbilical vein endothelial cells taken from newborns correlated with increased pulse wave velocity in the same children aged 9 years, which is considered a risk factor for future cardiovascular diseases [17].

Zhou S, et al. (2016, 2017) studied the methylation level of 24 CpG sites of *CDKN2A* gene (chr9: 21.993.993-21.995.909) and 12 CpG sites of *CDKN2B* gene (chr9: 22,008,804-22,009,259) in peripheral blood leukocytes of patients with ischemic stroke, depending on concomitant calcification of large vessels. In the first study, an increased methylation level of 1 CpG site of *CDKN2A* gene and 7 CpG sites of *CDKN2B* gene, as well as of the analyzed region as a whole, was found in patients with CA calcification [9]. In the second study, an increased methylation level of 4 CpG sites of *CDKN2A* gene and 11 CpG sites of *CDKN2B* gene in patients with aortic arch calcification was revealed [10]. At the same time, according to both studies,

Table 2

Methylation level of individual CpG sites in the enhancer region of *CDKN2A/2B* and *CDKN2B-AS1* genes in blood vessels and leukocytes in atherosclerotic lesions of the arteries

Tissue	CpG site methylation level, %, Q2 [Q1;Q3]		Source
	chr9:22,005,288 (cg19481686)	chr9:22,005,564 (cg08390209)	
Vessels			
Atherosclerotic CA (n=22)	53,85 [30,18; 63,57]	50,45 [32,24; 60,88]	Current study
Intact CA (n=18)	28,98 [24,19; 46,30]	26,31 [22,87; 36,78]	
Great saphenous veins (n=10)	40,90 [31,65; 43,00]	31,00 [28,57; 36,79]	
Atherosclerotic CA in patients with stroke (n=19)	55,03 [50,39; 61,24]	47,31 [43,50; 56,06]	GSE66500 [19]
Atherosclerotic CA in patients without stroke (n=19)	55,27 [49,70; 59,63]	50,04 [42,04; 53,88]	
Atherosclerotic coronary arteries (n=6)	69,13 [56,37; 78,95]	67,11 [57,87; 72,87]	GSE62867 [20]
Intact internal thoracic arteries (n=6)	73,20 [68,76; 73,57]	69,21 [67,30; 70,02]	
Intact great saphenous veins (n=6)	64,93 [59,79; 69,18]	63,18 [62,70; 65,00]	
Atherosclerotic aorta (n=15)	69,28 [66,95; 70,93]	66,52 [60,41; 71,18]	GSE46401 [21]
Intact aorta (n=15)	78,54 [77,12; 80,39]	81,34 [78,13; 85,01]	
Blood leukocytes			
Patients with severe CA atherosclerosis (n=22)	4,60 [2,84; 7,76]	7,47 [5,44; 8,77]	Current study
Healthy individuals (n=14)	6,11 [3,61; 12,25]	7,77 [5,92; 11,41]	
Patients with severe coronary atherosclerosis (n=8)	14,50 [9,04; 20,88]	15,54 [10,31; 18,84]	GSE107143 [22]
Healthy individuals (n=8)	12,85 [9,82; 14,58]	13,55 [11,24; 15,54]	
Patients with stroke due to atherosclerosis (n=132)	11,38 [9,65; 13,64]	12,50 [10,63; 14,23]	GSE69138 [23]
Patients with lacunar stroke (n=141)	11,51 [9,96;13,60]	12,98 [10,93; 14,27]	
Patients with cardioembolic stroke (n=127)	11,47 [11,51; 11,38]	12,20 [12,98; 12,50]	

Abbreviations: CA — carotid artery, Q1 — 25% quartile, Q2 — 50% quartile (median), Q3 — 75% quartile.

the average level of *CDKN2A* and *CDKN2B* methylation in peripheral blood leukocytes of patients with ischemic stroke did not exceed 6% [9, 10], which is generally consistent with this study.

Analysis of targeted bisulfite sequencing of amplicons using high-throughput parallel sequencing makes it possible to estimate the frequency of genotypes and their relationship with the DNA methylation level. In the region of GH09J022005 enhancer of *CDKN2A/2B* and *CDKN2B-AS1* genes, rs3217986:T>G is located (Figure 1). The G allele rs3217986 is associated with the risk of severe coronary atherosclerosis and myocardial infarction in two different populations [13, 14]. The distribution of rs3217986 genotypes in the patient group was as follows: 21 (95,5%) individuals with the TT genotype and 1 (4,5%) with the TG genotype, and in the control group — 13 (92,9%) individuals with the TT genotype and 1 (7,1%) with the TG genotype.

The relatively small sample size did not make it possible to accurately determine the relationship between the DNA methylation level in blood vessels and leukocytes, and SNP rs3217986:T>G and significant signs of atherosclerotic lesions of CA. The revealed difference in the DNA methylation level in the region of the enhancer of *CDKN2A/2B* and *CDKN2B-AS1* genes in CA may be associated not so much with their atherosclerotic lesion, but with the cellular heterogeneity of the vessels. To determine the functional significance of changes in DNA methylation level in cells and tissues, it is necessary to analyze the functional activity of genes. On the other hand, this work used modern technologies for analyzing the DNA methylation level in several types of tissues (vascular wall and peripheral blood leukocytes) in the same individuals. This approach takes into account the individual variability of DNA methylation. In addition, this study was the first to analyze the enhancer region of *CDKN2A/2B* and

CDKN2B-AS1 genes in vascular tissues and peripheral blood leukocytes of patients with clinically relevant atherosclerosis and healthy individuals.

Conclusion

In the present study, the relationship between an increase in the DNA methylation in the enhancer of the *CDKN2A/2B* and *CDKN2B-AS1* genes in CA and their atherosclerotic lesions was revealed, as well as the tissue-specific DNA methylation between vessels and peripheral blood leukocytes. Thus, the molecular genetic mechanisms of atherosclerotic arterial lesions should be studied using tissues and cells of arteries, and the search for biomarkers of this pathology, using peripheral blood leukocytes.

Relationships and Activities: none.

References

1. GWAS Catalog. Region: 9p21.3. https://www.ebi.ac.uk/gwas/regions/9p21.3. (9 August 2020).
2. Motterle A, Pu X, Wood H, et al. Functional analyses of coronary artery disease associated variation on chromosome 9p21 in vascular smooth muscle cells. Human molecular genetics. 2012;21(18)4021-9. doi:10.1093/hmg/dds224.
3. Almontashiri NAM. The 9p21.3 risk locus for coronary artery disease: A 10-year search for its mechanism. Journal of Taibah University Medical Sciences. 2017;12(3):199-204. doi:10.1016/j.jtumed.2017.03.001.
4. Dechamethakun S, Muramatsu M. Long noncoding RNA variations in cardiometabolic diseases. Journal of human genetics. 2017;62(1):97-104. doi:10.1038/jhg.2016.70.
5. Navarro E, Mallén A, Cruzado JM, et al. Unveiling ncRNA regulatory axes in atherosclerosis progression. Clinical and translational medicine. 2020;9(1):5. doi:10.1186/s40169-020-0256-3.
6. Aavik E, Babu M, Ylä-Herttuala S. DNA methylation processes in atherosclerotic plaque. Atherosclerosis. 2019;281:168-79. doi:10.1016/j.atherosclerosis.2018.12.006.
7. Clermont PL, Parolia A, Liu HH, et al. DNA methylation at enhancer regions: Novel avenues for epigenetic biomarker development. Frontiers in bioscience (Landmark edition). 2016;21:430-46. doi:10.2741/4399.
8. Nazarenko MS, Markov AV, Lebedev IN, et al. Methylation profile of INK4B-ARF-INK4A locus in atherosclerosis. Genetika. 2013;49(6):783-7. (In Russ.) doi:10.7868/s0016675813060076.
9. Zhou S, Cai B, Zhang Z, et al. CDKN2B Methylation and Aortic Arch Calcification in Patients with Ischemic Stroke. Journal of atherosclerosis and thrombosis. 2017;24(6):609-20. doi:10.5551/jat.36897.
10. Zhou S, Zhang Y, Wang L, et al. CDKN2B methylation is associated with carotid artery calcification in ischemic stroke patients. Journal of translational medicine. 2016;14(1):333. doi:10.1186/s12967-016-1093-4.
11. GeneCards®: The Human Gene Database. http://www.genecards.org.
12. MethPrimer. http://www.urogene.org/cgi-bin/methprimer2/MethPrimer.cgi.
13. Helgeland Ø, Hertel JK, Molven A, et al. The Chromosome 9p21 CVD- and T2D-Associated Regions in a Norwegian Population (The HUNT2 Survey). International journal of endocrinology. 2015;2015:164652. doi:10.1155/2015/164652.
14. Xu JJ, Jiang L, Xu LJ, et al. Association of CDKN2B-AS1 Polymorphisms with Premature Triple-vessel Coronary Disease and Their Sex Specificity in the Chinese Population. Biomedical and environmental sciences. 2018;31(11):787-96. doi:10.3967/bes2018.106.
15. Angeloni A, Bogdanovic O. Enhancer DNA methylation: implications for gene regulation. Essays Biochem. 2019;63(6):707-15. doi:10.1042/EBC20190030.
16. Aavik E, Lumivuori H, Leppänen O, et al. Global DNA methylation analysis of human atherosclerotic plaques reveals extensive genomic hypomethylation and reactivation at imprinted locus 14q32 involving induction of a miRNA cluster. European heart journal. 2015;36(16):993-1000. doi:10.1093/eurheartj/ehu437.
17. Murray R, Bryant J, Titcombe P, et al. DNA methylation at birth within the promoter of ANRIL predicts markers of cardiovascular risk at 9 years. Clinical epigenetics. 2016;8(1):90. doi:10.1186/s13148-016-0259-5.
18. Stary HC, Chandler AB, Dinsmore RE, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arteriosclerosis, thrombosis, and vascular biology. 1995;15(9):1512-31. doi:10.1161/01.atv.15.9.1512.
19. Zaina S, Gonçalves I, Carmona FJ, et al. DNA methylation dynamics in human carotid plaques after cerebrovascular events. Arteriosclerosis, thrombosis, and vascular biology. 2015;35(8):1835-42. doi:10.1161/ATVBAHA.115.305630.
20. Nazarenko MS, Markov AV, Lebedev IN, et al. A comparison of genome-wide DNA methylation patterns between different vascular tissues from patients with coronary heart disease. PLoS One. 2015;10(4):e0122601. doi:10.1371/journal.pone.0122601.
21. Zaina S, Heyn H, Carmona FJ, et al. DNA methylation map of human atherosclerosis. Circulation. Cardiovascular genetics. 2014;7(5):692-700. doi:10.1161/CIRCGENETICS.113.000441.
22. Istas G, Declerck K, Pudenz M, et al. Identification of differentially methylated BRCA1 and CRISP2 DNA regions as blood surrogate markers for cardiovascular disease. Scientific reports. 2017;7(1):5120. doi:10.1038/s41598-017-03434-0.
23. Soriano-Tárraga C, Jiménez-Conde J, Giralt-Steinhauer E, et al. Epigenome-wide association study identifies TXNIP gene associated with type 2 diabetes mellitus and sustained hyperglycemia. Human molecular genetics. 2016;25(3):609-19. doi:10.1093/hmg/ddv493.

https://russjcardiol.elpub.ru
doi:10.15829/1560-4071-2020-3978

ISSN 1560-4071 (print)
ISSN 2618-7620 (online)

Expression of matrix metalloproteinases 1, 2, 9, 12 in xenogenic tissues of epoxy-crosslinked bioprosthetic heart valves explanted due to dysfunction

Kostyunin A. E., Glushkova T. V.

Aim. To study the expression of matrix metalloproteinases (MMPs) 1, 2, 9, 12 in the leaflets of epoxy-crosslinked bioprosthetic heart valves (BHVs) explanted due to dysfunction and to study the possibility to accumulate these enzymes in xenogenic tissues.

Material and methods. We examined 19 leaflets of 7 mitral and aortic BHVs removed during re-replacement. Tissue sections for microscopy were prepared using a cryotome. Cellular typing and detection of MMP 1, 2, 9, 12 expression in samples were performed using immunohistochemical staining with antibodies to PTPRC/CD45, CD68, myeloperoxidase, and the corresponding MMPs. Analysis of samples was performed by light microscopy.

Results. In 17 studied leaflets from 6 explanted BHVs, sporadic infiltrates consisting of macrophages (PTPRC/CD45+, CD68+) were revealed. A positive staining for MMP 1, 2, 9, 12 was colocalized with immune cell infiltrates. Also, positive staining was observed without cell infiltration. The pericardial BHV removed due to thrombosis 2 days after implantation did not show signs of macrophage infiltration or MMP expression in xenogenic tissues, but the thrombus stained positive for MMP-9 and included a large number of neutrophils positive for myeloperoxidase.

Conclusion. Macrophages and other immune cells infiltrating xenogenic tissues of epoxy-crosslinked BHV are sources of MMPs 1, 2, 9, 12. In addition, MMP-9 can diffuse into BHV leaflets from the blood plasma of patients. The deposition of MMP may contribute to rupture and calcification of the leaflets leading to the implant dysfunction.

Key words: bioprosthetic heart valves, structural valve degeneration, cell infiltration, matrix metalloproteinases.

Relationships and Activities. This study was carried within the program of basic research on the fundamental subject of Research Institute for Complex Issues of Cardiovascular Diseases № 0546-2019-0002 “Pathogenetic rationale for the development of implants for cardiovascular surgery based on biocompatible materials, with the implementation of a patient-centered approach using mathematical modeling, tissue engineering methods and genomic predictors”.

Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russia.

Kostyunin A. E.* ORCID: 0000-0001-6099-0315, Glushkova T. V. ORCID: 0000-0003-4890-0393.

*Corresponding author:
Rhabdophis_tigrina@mail.ru

Received: 22.06.2020
Revision Received: 04.08.2020
Accepted: 06.08.2020

For citation: Kostyunin A. E., Glushkova T. V. Expression of matrix metalloproteinases 1, 2, 9, 12 in xenogenic tissues of epoxy-crosslinked bioprosthetic heart valves explanted due to dysfunction. *Russian Journal of Cardiology*. 2020;25(10):3978. (In Russ.) doi:10.15829/1560-4071-2020-3978

Today, heart valve prosthesis are the main method of radical treatment of heart defects [1, 2]. More than 200,000 such operations are performed annually in the world, and according to forecasts, by 2050, their number will increase to 850,000, which is associated with the population ageing in developed countries, and therefore, which means an increase in the prevalence of heart valve diseases [3]. Mechanical or xenogeneic bioprostheses (BP) of heart valves are most often used. The latter are made from animal derived tissues stabilized with glutaraldehyde (GA) or epoxides [4]. Optimal hemodynamic parameters and low thrombogenicity distinguish biological from mechanical prosthesis [1, 2]. However, even modern BP are prone to structural degeneration within 10-15 years after implantation, which limits their use in surgical practice [5].

The processes underlying the structural degeneration of BP are poorly understood. The data of modern studies suggest that they may be related on immune mechanisms, partly reminiscent of those involved in the transplant rejection and the development of atherosclerosis [6, 7]. The results of a number of original studies demonstrate the presence of dense macrophage infiltrates co-localized with areas of degenerated biological tissue in the explanted GA-fixed BP [8, 9]. Cells infiltrating BP have been shown to produce matrix metalloproteinases (MMPs) [8, 9]. The latter are zinc-related proteolytic enzymes that catalyze the cleavage of extracellular matrix (ECM) proteins, such as collagens and elastin [10]. Potentially, the deposition of MMPs in BP can contribute to the structural destruction of the prosthetic xenomaterial and the development of hemodynamically significant obstruction or regurgitation caused by calcification or rupture of the leaflets. The data of *in vivo* experiments, indicating that elastolysis promotes the calcification of biological tissue [11], confirm this assumption. Also, high levels of MMP-2 and especially MMP-9 were noted in the tissues of GA-fixed BP, explanted due to rupture [9].

Despite the potentially important role of MMPs in BP degeneration, a limited number of studies have been devoted to the expression of these enzymes in implant tissues [8, 9]. All MMPs present in BP tissues is still unknown. Possible ways of their accumulation in prosthetic valves are also poorly understood. There are no data on the presence of MMPs and their role in the degeneration of epoxy-treated BP in the literature. Thus, the aim of the present study was to assess the expression of MMP-1/-2/-9/-12 in xenogenic tissues of epoxy-treated BP explanted due to dysfunction and to identify related patterns. These MMPs were selected due to the fact that they are the most studied enzymes of the considered family, which are responsible for pathological remodeling of the leaflets' ECM [12].

Material and methods

The material was epoxy-treated mitral or aortic BPs (NeoKor, Russia) removed from 7 patients during re-replacement performed in 2019-2020. A total of 19 leaflets of 7 explanted BPs were examined. Among the latter, 4 samples were represented by xenogenic aortic BPs (KemCor (n=2); PeriCor (n=2)), 3 — pericardial (UniLine (n=2) and TiAra (n=1)). The mean age of patients at primary surgery was 57±11 years. The mean period of BP functioning was 12±8 years, except for the pericardial BP, which was excised 2 days after implantation due to thrombosis. The study was approved by the local ethics committee. All patients signed informed consent.

The material obtained during reoperations after macroscopic analysis was frozen at a temperature of -140° C. To study the cellular infiltration and expression of MMPs, serial sections of 7±1 μm were prepared using a Microm HM 525 Cryostat (Thermo Scientific, Germany). When making sections from each BP, the central part of 2-3 valves from the basis to the free edge was used, as well as areas with degenerative changes. Before staining, the sections were fixed for 10 min at room temperature with 4% paraformaldehyde solution, followed by three times washing (5 min each) in phosphate-buffered saline (PBS) (pH 7,4) on a shaker (Polymax 1040, Heidolph, 25 rpm). Cell typing (markers: PTPRC/CD45, CD68, and myeloperoxidase) and detection of MMP-1/-2/-9/-12 were performed by manual immunohistochemical staining, for which the corresponding primary antibodies (Abcam PLC, UK) were used (Table 1). Immunohistochemical reaction was performed using the NovoLink Polimer Detection System kit (RE7150-CE, Leica Microsystems Inc., USA) according to the manufacturer's modified protocol. First, endogenous peroxidase was blocked with 4% hydrogen peroxide solution (Peroxidase Block) for 5 min. Then the sections were washed twice in PBS and the nonspecific binding of antibodies with 0,4% casein saline solution with ancillary reagents (Protein Block) was blocked for an hour. Primary antibodies were diluted according to the manufacturer's protocol in 1% saline solution of bovine serum albumin. The dilutions used are shown in Table 1. Sections were incubated with antibodies for 20 hours in a closed box at +4° C. Then, the sections were washed three times in PBS and incubated with anti-rabbit antibodies (Novolink Polymer). After three-times washing in PBS, the sections were treated with 0,087% diaminobenzidine solution for 2 min. Then the sections were washed with double-distilled water and placed in hematoxylin for 10 min. Then, the sections were blued in running water (5 min), dehydrated in three changes of 95% ethanol (5 min each) and cleared in 3 changes of xylene (5 min each).

Table 1

Primary antibodies used in the study

Antigens used	Antibody reference numbers	Appointment	Dilution used
CD45	ab10558	Detection of PTPRC/CD45 (pan-leukocyte marker). Identification of immune cells in the BP.	1:3000
CD68	ab227458	Detection of CD68 (macrophage marker). Assessment of BP macrophage infiltration.	1:500
Myeloperoxidase	ab208670	Detection of myeloperoxidase (neutrophil marker). Assessment of the acute inflammatory response to BP.	1:1500
MMP-1	ab52631	Detection of MMP-1/-2/-9/-12. Revealing MMP expression in BP xenogenic tissues.	1:1000
MMP-2	ab92536		
MMP-9	ab38898		
MMP-12	ab52897		

Abbreviations: BP — heart valve bioprostheses, MMP — matrix metalloproteinases.

Samples with immunohistochemical stains were analyzed using an AxioImager.A1 light microscope (Zeiss, Germany). The images were processed using the AxioVision software (Zeiss, Germany).

Intact epoxy-treated xenogenic tissues were used as controls: porcine aortic valve leaflets and bovine pericardium. Also, one section with negative control of primary and secondary antibodies was isolated on each slide.

Results

Macroscopic analysis of the explanted BPs. The xenogenic aortic BPs included in the study had signs of primary tissue failure in the form of leaflet breaks in the commissural region, perforations, and calcification. The reason for re-replacement for these BPs was regurgitation. It is worth noting that the calcifications were insignificant.

Xenogenic pericardial BPs showed no signs of rupture and perforation of the leaflets. One of the three prostheses showed insignificant calcification. In one case, early thrombosis of BP was the cause of reoperation.

Characteristics of cellular infiltration of BPs. Infiltration of xenogenic material by the recipient's cells was detected in 17 leaflets taken from 6 explanted BPs that functioned for 2,5-25 years. In the studied samples, sporadic cellular infiltrates were noted, localized mainly on the surface or in the presurface layers of the xenogenic ECM near the base of the valves. Greater cellular infiltration was noted from the side of the excretory section. In turn, no signs of infiltration of the recipient's cells were found in the two studied valves, taken from the pericardial BP removed due to thrombosis 2 days after implantation. At the same time, the presence of a large number of cells in the thrombus. Aggressive cellular infiltration associated with the penetration of cells deep into the xenogenic material was detected only in the leaflets of aortic BPs, in the presence of large calcifications, perforations, or areas with pronounced dissociation of ECM, near which significant cell clusters were concentrated.

According to the results of immunohistochemical staining, it was found that the majority of cells infiltrating the prosthetic xenogenic biomaterial express PTPRC/CD45 and CD68 (Figure 1). In the leaflets of BP, which functioned for 2 days, these markers were not detected, but the cells in the thrombus were positive for myeloperoxidase (Figure 2). Cells positively stained with anti-myeloperoxidase antibodies were not detected in other samples.

Expression of MMPs in xenogenic tissues of explanted BPs. Immunohistochemical staining of sections with anti-myeloperoxidase antibodies (MMP-1/-2/-9/-12) revealed the studied enzymes in all BP leaflets, with the exception of samples taken from an implant that functioned for two days. A positive staining for MMP-1/-2/-12 was observed exclusively near cell infiltrates, while staining for MMP-9 was observed both in colocalization with cells and in acellular ECM (Figure 3). It is important to note that the intensity of MMP-9 staining did not depend on the presence of recipient cells in the samples and remained high even in their complete absence. The most intense MMP-9 staining was observed for areas with loose tissue, as well as for spongiosa of xenogenic aortic BPs (Figure 4). The leaflets of xenogenic BPs stained for MMP-9 more intensely than those of the pericardial BPs. In turn, the xenogenic biomaterial functioned for two days did not show the expression of the studied enzymes, but the thrombus formed on its surface showed positive staining for MMP-9.

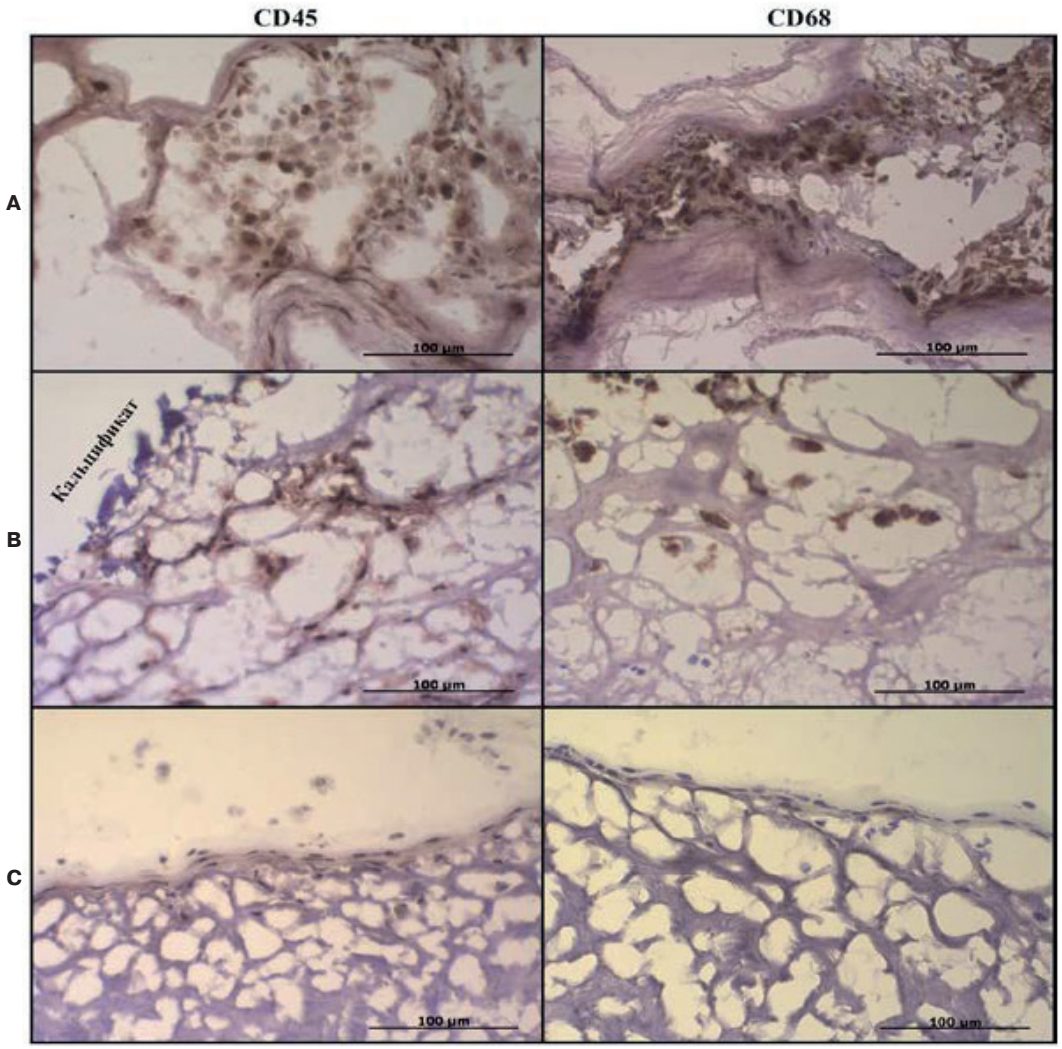


Figure 1. Results of immunohistochemical staining of samples for PTPRC/CD45 and CD68. The densest cellular infiltrates were noted in the loosened surface layers of leaflet ECM in xenogenic aortic BPs (**A**), while inside there were small groups of immune cells located near the calcifications (**B**). No infiltration of the recipient's cells into the depths of the xenogenic pericardial BPs was revealed: cell clusters were present only on the surface of the valves and had a weak staining for the studied markers (**C**).

In all controls, positive staining for PTPRC/CD45, CD68 and myeloperoxidase, as well as MMP-1/-2/-9/-12, was not detected.

Discussion

The results of this study are consistent with the data obtained by other authors on GA-treated BPs [8, 9]. Majority of cells were positive for PTPRC/CD45 and CD68, which indicates macrophage infiltration of epoxy-treated implants and indicates their chronic immune rejection [7]. The leaflets of BP functioned for two days did not show macrophages but contained thrombotic masses with included neutrophils on their surface, which indicates an acute inflammatory response that occurs in the first days upon implantation of any foreign body [13]. Based on the data on staining with anti-myeloperoxidase antibodies, it can be concluded that the main source of MMP-1/-2/-12 are macrophages, while MMP-9 mainly diffuses from the blood plasma. It is important to note that the tendency associated with the accumulation of MMP-9 from plasma was revealed for the first time in xenogenic BPs. Differences in the color intensity for MMP-9 may indicate that the leaflets of xenogenic aortic BPs are characterized by a more pronounced diffusion of this enzyme as compared to pericardial BPs. Apparently, this pattern is due to the looser structure of the ECM of porcine aortic valve leaflets, formed as a result of glycosaminoglycan loss, which are not stabilized by GA and diepoxy compounds [14]. In turn, the bovine pericardium retains a dense structure even after treatment with preservatives, which probably prevents the diffusion

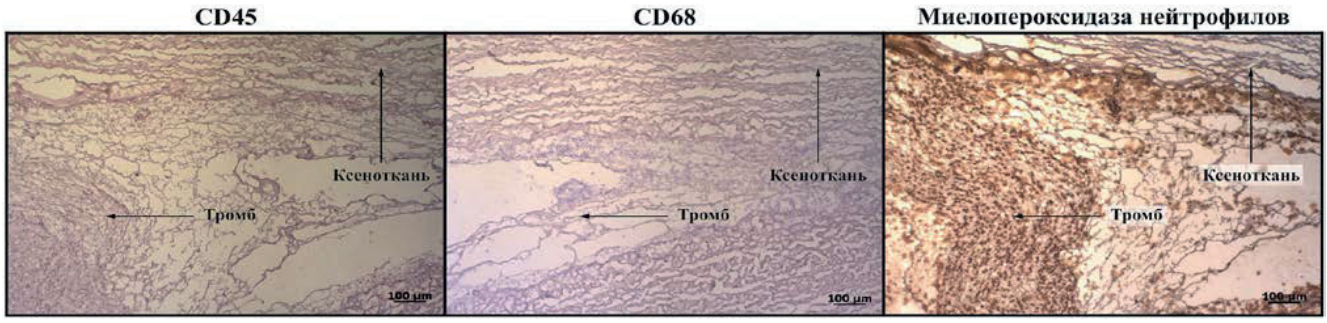


Figure 2. Immunohistochemical staining with anti-myeloperoxidase antibodies of BP leaflets, removed 2 days after implantation, shows the presence of numerous neutrophils in the thrombus formed on the surface of leaflets. At the same time, no cells positive for PTPRC/CD45 and CD68 were found in these samples.

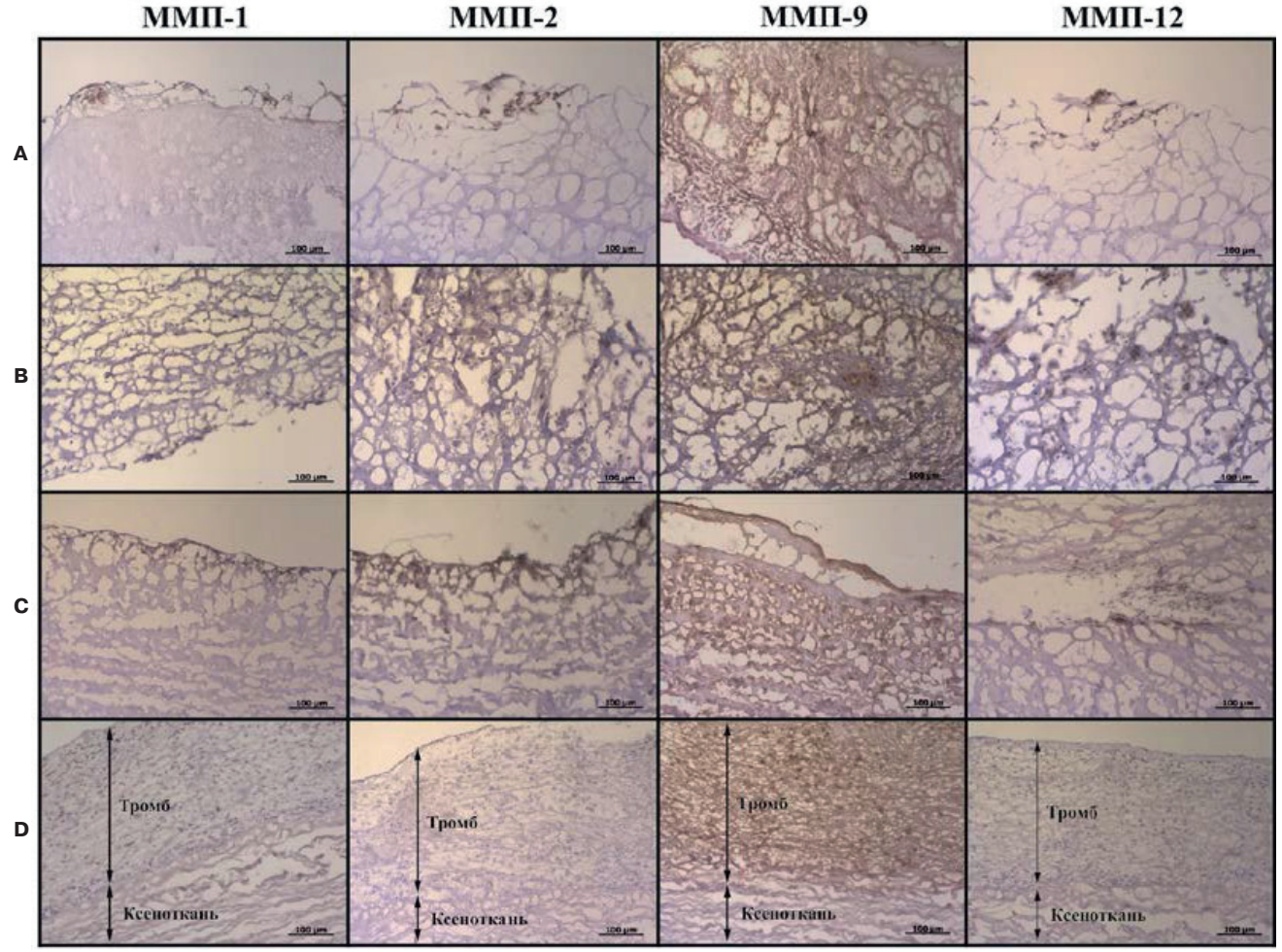


Figure 3. Results of immunohistochemical staining of samples for MMP-1/-2/-9/-12. MMP-1/-2/-12 are colocalized with the cells of the recipient. This pattern is clearly seen in the example of both weakly and strongly infiltrated by cells of leaflets of xenogenic aortic (**A**) (**B**) and pericardial (**C**) prostheses. In turn, staining for MMP-9 is almost independent of the presence of cells in the valves (**A**, **B**, and **C**). This suggests that the main source of MMP-9 is not cells, but the blood plasma of patients. The absence of expression of all MMPs in the xenogenic tissues of the prosthesis removed 2 days after implantation due to thrombosis (**D**), but intense staining of the thrombotic mass on its surface with anti-myeloperoxidase antibodies (MMP-9), confirms the hypothesis of the impregnation of leaflets with this enzyme.
Abbreviation: MMP — matrix metalloproteinases.

of substances from the surrounding liquids. It is noteworthy that GA-treated xenogenic pericardial BPs often require replacement due to calcification-related stenosis, while xenogenic aortic BPs are more prone to leaflet rupture [15]. In the present study, ruptures and perforations of leaflets were also noted for epoxy-treated xenogenic aortic BPs, which was not observed in pericardial BPsD. In

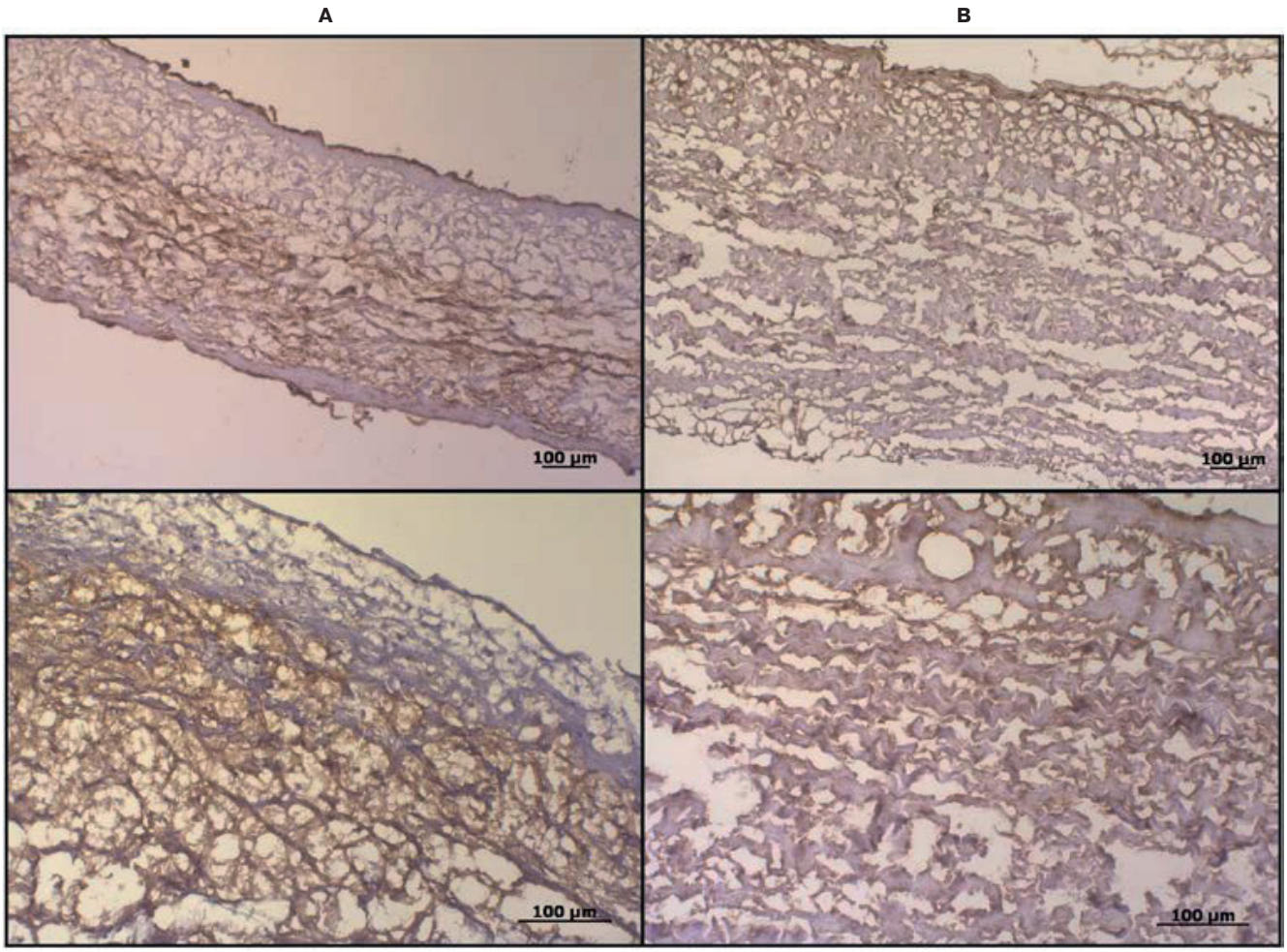


Figure 4. The intensity of staining for MMP-9 is related to the tissue density: the denser and more structured the matrix, the weaker the enzyme signal. This is noticeable when comparing the valves of xenogenic aortic (**A**) and pericardial prostheses (**B**), especially their most intensely stained areas. In the leaflets of xenogenic aortic prostheses, the spongiosa is stained most intensively for MMP-9.

part, this can be explained by the more intensive accumulation of proteolytic enzymes from the blood plasma by the tissues of the latter, followed by the cleavage of collagen fibers of ECM.

Study limitations. The xenogenic aortic and pericardial BPS used in the study are not comparable in terms of the functioning duration and the causes of dysfunctions. The former functioned for 15±6,5 years, the latter for 4±2 years. Thus, more aggressive cellular infiltration and more pronounced staining for MMP-9 of xenogenic aortic BPs as compared to pericardial BPs may be the result not of structural differences in their ECM. More severe tissue wear may be caused by a longer period of functioning. Further studies on comparable samples are needed to confirm the results.

Conclusion

Macrophages and other immune cells infiltrating xenogenic tissues of epoxy-treated BPs are sources of MMPs 1, 2, 9, 12. At the same time, the expression of MMPs is different: MMP-1/-2/-12 are localized exclusively near cell clusters, while high levels of MMP-9 can be detected even in the absence of cell infiltration. This observation suggests that MMP-9 diffuses into xenogenic biomaterial from the blood plasma of patients. The deposition of MMP may contribute to rupture and calcification of the leaflets leading to the implant dysfunction.

Relationships and Activities. This study was carried within the program of basic research on the fundamental subject of Research Institute for Complex Issues of Cardiovascular Diseases № 0546-2019-0002 “Pathogenetic rationale for the development of implants for cardiovascular surgery based on biocompatible materials, with the implementation of a patient-centered approach using mathematical modeling, tissue engineering methods and genomic predictors”.

References

1. Baumgartner H, Falk V, Bax JJ, et al. 2017 ESC/EACTS Guidelines for the management of valvular heart disease. Eur. Heart J. 2017;38(36):2739-91. doi:10.1093/eurheartj/ehx391.
2. Nishimura RA, Otto CM, Bonow RO, et al. 2014 AHA/ACC guideline for the management of patients with valvular heart disease: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J. Am. Coll. Cardiol. 2014;63(22):2438-88. doi:10.1016/j.jacc.2014.02.537.
3. Bax JJ, Delgado V. Bioprosthetic heart valves, thrombosis, anticoagulation, and imaging surveillance. JACC Cardiovasc. Interv. 2017;10(4):388-90. doi:10.1016/j.jcin.2017.01.017.
4. Kudryavtseva YuA. Bioprosthetic heart valves. From idea to clinical use. Complex Issues of Cardiovascular Diseases. 2015;4:6-16. (In Russ.) doi:10.17802/2306-1278-2015-4-6-16.
5. Capodanno D, Petronio AS, Prendergast B, et al. Standardized definitions of structural deterioration and valve failure in assessing long-term durability of transcatheter and surgical aortic bioprosthetic valves: a consensus statement from the European Association of Percutaneous Cardiovascular Interventions (EAPCI) endorsed by the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS). Eur. Heart J. 2017;38(45):3382-90. doi:10.1093/eurheartj/ehx303.
6. Cote N, Pibarot P, Clavel MA. Incidence, risk factors, clinical impact, and management of bioprosthesis structural valve degeneration. Curr. Opin. Cardiol. 2017;32(2):123-9. doi:10.1097/HCO.0000000000000372.
7. Manji RA, Ekser B, Menkis AH, et al. Bioprosthetic heart valves of the future. Xenotransplantation. 2014;21(1):1-10. doi:10.1111/xen.12080.
8. Shetty R, Pibarot P, Audet A, et al. Lipid-mediated inflammation and degeneration of bioprosthetic heart valves. Eur. J. Clin. Invest. 2009;39(6):471-80. doi:10.1111/j.1365-2362.2009.02132.x.
9. Simionescu A, Simionescu DT, Deac RF. Matrix metalloproteinases in the pathology of natural and bioprosthetic cardiac valves. Cardiovasc. Pathol. 1996;5(6):323-32.
10. Cui N, Hu M, Khalil RA. Biochemical and biological attributes of matrix metalloproteinases. Prog. Mol. Biol. Transl. Sci. 2017;147:1-73. doi:10.1016/bs.pmbts.2017.02.005.
11. Bailey M, Xiao H, Ogle M, et al. Aluminum chloride pretreatment of elastin inhibits elastolysis by matrix metalloproteinases and leads to inhibition of elastin-oriented calcification. Am. J. Pathol. 2001;159(6):1981-6. doi:10.1016/S0002-9440(10)63048-9.
12. Kostyunin AE, Yuzhalin AE, Ovcharenko EA, et al. Development of calcific aortic valve disease: do we know enough for new clinical trials? J. Mol. Cell. Cardiol. 2019;132:189-209. doi:10.1016/j.yjmcc.2019.05.016.
13. Mariani E, Lisignoli G, Borzì RM, et al. Biomaterials: foreign bodies or tuners for the immune response? Int. J. Mol. Sci. 2019;20(3):pii:E636. doi:10.3390/ijms20030636.
14. Rezvova MA, Kudryavceva YuA. Modern approaches to protein chemical modification in biological tissue, consequences and application. Bioorganicheskaia khimiia. 2017;44(1):1-16. (In Russ.) doi:10.7868/S0132342318010025.
15. Arsalan M, Walther T. Durability of prostheses for transcatheter aortic valve implantation. Nat. Rev. Cardiol. 2016;13(6):360-7. doi:10.1038/nrcardio.2016.43.

https://russjcardiol.elpub.ru
doi:10.15829/1560-4071-2020-4102

ISSN 1560-4071 (print)
ISSN 2618-7620 (online)

Mixed cardiomyopathy associated with a *DSP* gene variant: a case report and literature review

Shlyk I. V.[1], Streltsova A. A.[2], Teplov V. M.[1], Gavrilova E. G.[1], Kulikov A. N.[1,3], Krutikov A. N.[2], Rybakova M. G.[1], Kuznetsova I. A.[1], Andreeva S. E.[2], Bezhanishvili T. G.[1], Khudyakov A. A.[2], Kostareva A. A.[1,2], Gudkova A. Ya.[1,2]

A case report of mixed cardiomyopathy (combination of non-compaction cardiomyopathy and arrhythmogenic right ventricular dysplasia) associated with a *DSP* gene variant is presented. The first and only symptom of the disease was sudden cardiac death.

Key words: sudden cardiac death, long QT syndrome, myocardial electrical instability, arrhythmogenic cardiomyopathy, non-compaction cardiomyopathy, genetic variants *DSP* and *KCNH2*.

Relationships and Activities. This work was supported by the Russian Science Foundation grant 20-15-00271.

[1]First Pavlov State Medical University of St. Petersburg, St. Petersburg; [2]Almazov National Medical Research Center, St. Petersburg; [3]Kirov Military Medical Academy, St. Petersburg, Russia.

Shlyk I. V. ORCID: 0000-0003-0977-8081, Streltsova A. A. ORCID: 0000-0002-2766-8946, Teplov V. M. ORCID: 0000-0002-4299-4379, Gavrilova E. G. ORCID: 0000-0002-9126-3206, Kulikov A. N. ORCID: 0000-0002-4544-2967, Krutikov A. N. ORCID: 0000-0001-6897-6171, Rybakova M. G. ORCID: 0000-0002-8404-1859, Kuznetsova I. A. ORCID: 0000-0003-4368-7810, Andreeva S. E.* ORCID: 0000-0001-7306-9525, Bezhanishvili T. G. ORCID: 0000-0002-3167-6340, Khudyakov A. A. ORCID: 0000-0001-9214-0868, Kostareva A. A. ORCID: 0000-0002-9349-6257, Gudkova A. Ya. ORCID: 0000-0003-0156-8821.

*Corresponding author:
andreeva.sofi2012@yandex.ru

Received: 09.09.2020
Revision Received: 23.09.2020
Accepted: 28.09.2020

For citation: Shlyk I. V., Streltsova A. A., Teplov V. M., Gavrilova E. G., Kulikov A. N., Krutikov A. N., Rybakova M. G., Kuznetsova I. A., Andreeva S. E., Bezhanishvili T. G., Khudyakov A. A., Kostareva A. A., Gudkova A. Ya. Mixed cardiomyopathy associated with a *DSP* gene variant: a case report and literature review. *Russian Journal of Cardiology*. 2020;25(10):4102. (In Russ.) doi:10.15829/1560-4071-2020-4102

Sudden cardiac death (SCD) is one of the leading causes of death in developed countries. SCD in young people is a significant aspect of public health, which incidence (excluding infants) ranges from 0,6 to 6,2 per 100,000 [1]. In the overwhelming majority of cases (85%), the mechanism of SCD is electric myocardial instability, leading to ventricular tachyarrhythmias — ventricular tachycardia and ventricular fibrillation (VF), followed by asystole [2]. According to recent data, the main causes of SCD in people under 35 years of age include arrhythmogenic cardiomyopathy (ACM), channelopathies, hypertrophic cardiomyopathy, left ventricular noncompaction cardiomyopathy (LVNC), coronary artery anomalies and myocarditis [3-5].

According to the 2019 Heart Rhythm Society expert consensus statement, ACM is a hereditary myocardial disease characterized by progressive fibro-fatty replacement of the myocardium involving not only the right ventricle, but also the left ventricle (LV), which can have biventricular forms and clinically manifests as ventricular arrhythmias with a high risk of SCD. Currently, the left ventricular ACM is less studied [5-7].

The prevalence of the disease (depending on the region) is 1:2000-1:5000 [8]. The debut most often falls on the second and third decades of life [6]. According to molecular genetic studies, ACP is most often associated with mutations in genes encoding intercellular junction proteins: desmoplakin (*DSP*), desmoglein-2 (*DSG2*), desmocollin-2 (*DSC2*), plakophyllin-2 (*PKP2*), plakoglobin (*JUP*), desmin (*DES*), and a number of other proteins [9, 10]. The expressiveness and penetrance of clinical and morphological signs in ACM are extremely variable. Factors such as sex, age, physical activity, and chronic intoxication play an important role in the formation of the phenotype [11]. In approximately 3-6% of patients, the cause of disease is the presence of more than one pathogenic or possible pathogenic genetic variant that contributes to the development of the disease phenotype [6]. Patients with ACM associated with the carriage of two or more pathogenic variants, as a rule, have an earlier age of onset and a progressive course, including with a high risk of SCD [6]. At autopsy, 20% of those who die suddenly at the age of 35 years show signs of arrhythmogenic right ventricular dysplasia (ARVD) [12]. In St. Petersburg, according to Gordeeva M. V. et al. (2012), ARVD is a frequent (14,1%) cause of SCD in young people [13].

In the study by Lutokhina Yu.A. et al. (2018) with an average follow-up of 13,5 [4; 34] months, 4 clinical forms of ACM were identified: latent arrhythmic (50%), manifest arrhythmic (20%), ARVD with a with predominant biventricular chronic heart failure (16%) and ARVD in combination with LVNC (14%) [11].

Of interest is the combination of ACM and LVNC, which is also an independent morphological and functional phenotype of LVNC (Table 1).

In the 2006 American Heart Association classification, both ACM and LVNC are defined as primary genetic cardiomyopathies (CM) [4]. The classifications of clinical course of ACMP proposed by Fontaine G in 1995 and 1998 have not found wide practical application for selecyion of management, treatment and prevention of SCD [14, 15]. According to 2008 European Society of Cardiology classification, ACM was identified as a separate type of CM, while LVNC refers to an unclassified CM [5]. At the same time, the separation of mixed morphological and functional phenotypes of CM is the subject of study. This approach is an advantage of the 2013 MOGE(S) classification system [16-18].

The variety of genes associated with non-compaction cardiomyopathy (NCC) suggests its phenotypic heterogeneity. By now, ideas about the genetic basis of myocardial non-compaction have expanded, not only as a concomitant phenotype in genetic CM, but also as a component of other heart diseases — congenital heart defects and some orphan diseases [4, 19]. The phenotype of myocardial non-compaction can be formed under the influence of coexisting multiple genetic variants [20], determined by epigenetic and environmental factors [21].

There are 9 different morphofunctional phenotypes (Table 1) [6].

Differential diagnosis in NCC is in some cases extremely difficult due to its phenotypic heterogeneity.

This article presents a clinical case of SCD in a 21-year-old woman with mixed CMP (NCC+ACM), complicated by VF and myocardial infarction (MI).

Case report

Twenty-one-year-old female patient suddenly lost consciousness. The collection of medical history was carried out with relatives. Before this case, the patient did not seek medical help. Intake of drugs and alcohol was ruled out.

Table 1

Morphofunctional phenotypes of NCC [6]

1. LV non-compaction 2. RV non-compaction 3. Biventricular noncompaction 4. NCC+DCM 5. NCC+HCM 6. NCC+RCM	7. Mixed form: — NCC+HCM+DCM or — NCC+DCM+RCM 8. NCC+CHD 9. NCC+ACM

Abbreviations: ACM — arrhythmogenic cardiomyopathy, CHD — congenital heart disease, HCM — hypertrophic cardiomyopathy, DCM — dilated cardiomyopathy, LV — left ventricle, NCC — non-compaction cardiomyopathy, RV — right ventricle, RCM — restrictive cardiomyopathy.

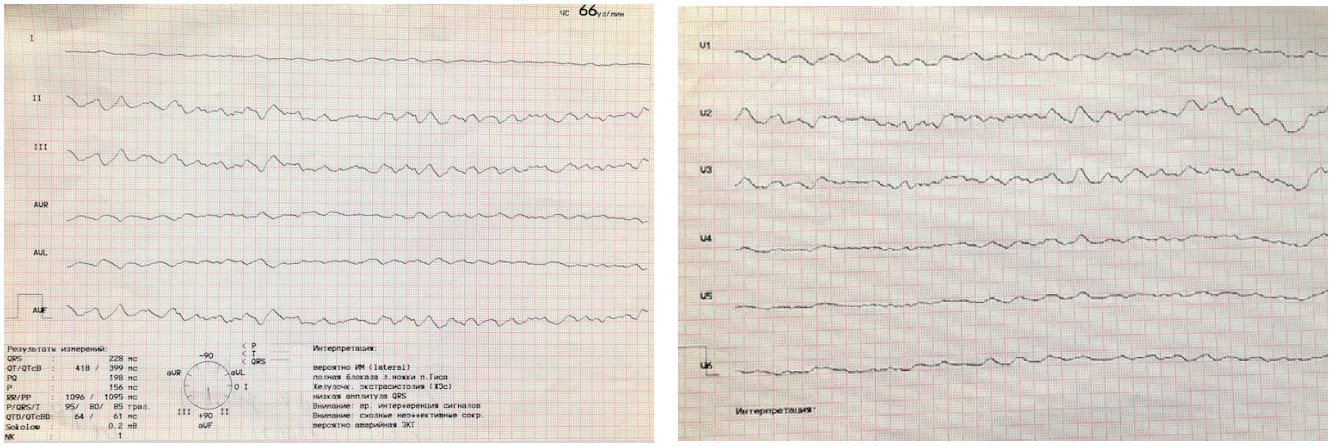


Figure 1. Cardiac arrest (VF). Paper speed: 50 mm/sec.

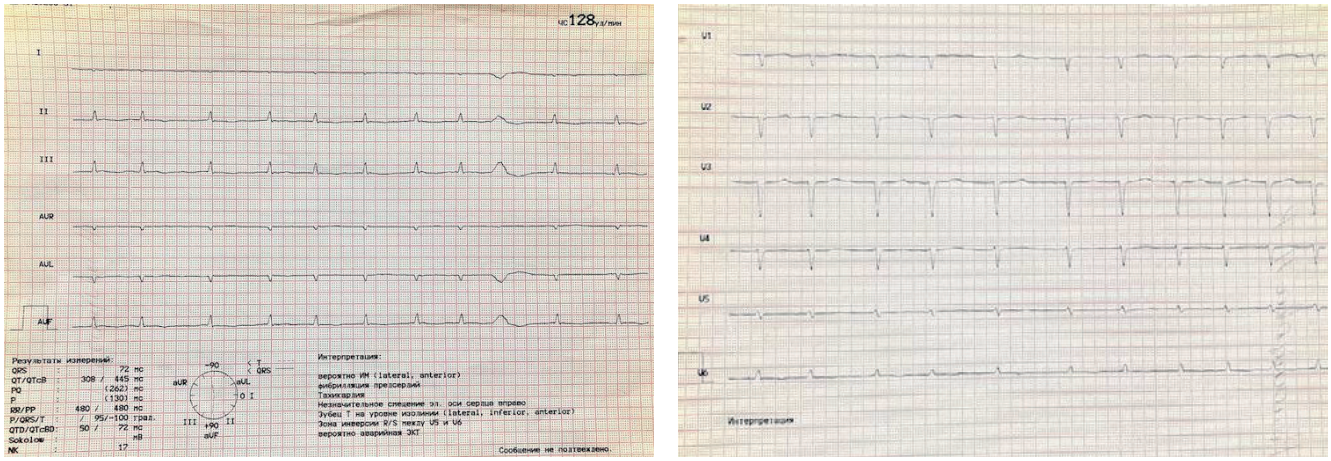


Figure 2. ECG of a 21-year-old female patient with mixed CMP: NCC and arrhythmogenic dysplasia associated with genetic DSP and KCNH2 variants. Paper speed: 50 mm/sec.

Collection of a genealogical history revealed that the father suddenly died at the age of 35 (no medical information).

At the time of ambulance arrival, the patient's condition was very ill — stage II-III coma, areflexia. Spontaneous breathing, central pulse. Blood pressure were not determined. Cardiopulmonary resuscitation was immediately started.

Electrocardiography (ECG) recorded the VF (Figure 1). Defibrillation, intravenous infusion of amiodarone, tracheal intubation and artificial lung ventilation with the Drager Oxylog system was carried out. Lucas II chest compression system was used.

An emergency implantation of an extracorporeal membrane oxygenation (ECMO) system was performed. Against the background of intensive therapy, atrial fibrillation (AF) was recorded with a ventricular rate of 83 bpm (RR, 0,72 sec; QRS, 0,07 sec). Right axis deviation, transition zone between V4 and V5, non-rise of r wave from V1 to V4 (rS). Diffuse repolarization changes presented as weak negative and weak positive T waves. Attention was drawn to the decrease in the QRS voltage (Figure 2).

Cardiac monitoring revealed a transient QT prolongation up to 0,440 sec. According to Bazett's formula, QT_{corr} was 0,518 sec.

Due to technical difficulties (extracorporeal membrane oxygenation), it was not possible to obtain a high-quality image with echocardiography. At the time of admission, there were no significant structural cardiac changes (Figure 3A); increased LV trabecularity was noted (Figure 3B). A repeated examination performed at the end-stage disease showed a significant decrease in global LV contractility (LV ejection fraction, 20%) due to severe hypokinesia, asynchronous LV contraction. The severity of the condition did not allow for cardiac magnetic resonance imaging.

According to laboratory tests, a significant increase in the venous troponin I level (⩾33,9 ng/ml) was revealed [22]. MI was suspected. Coronary angiography (CA) did not reveal any significant pathology.

Pulmonary angiography also did not reveal structural changes. Pulmonary artery pressure was within the normal range (13-15/1-3 mm Hg). According to angiography, carotid, vertebral arteries and their intracranial branches had no abnormalities.

The absence of significant atherosclerotic lesions of epicardial coronary arteries did not rule out the suspected type 2 MI within the unknown CM [23-25].

The severity of the patient's condition was specified by postresuscitation syndrome, an increase in multiple organ failure: hepatic, renal, respiratory, cerebral (anoxic brain damage), Killip IV acute heart failure. Despite the ongoing intensive therapy, after 4 days the patient died without recovering consciousness.

Post-mortem examination

The central changes were found in the cardiovascular system. Other systems and organs were without significant findings.

The heart is cone-shaped with a pointed apex measuring 12,0x10,0x6,5 cm, weighing 220 g; cardiac index — 0,003 (Figure 4A).

Epicardium of uneven thickness with areas of thinning or growth of adipose tissue.

Valvular endocardium. The *mitral valve leaflets* are with myxomatic transformation, dome-shaped bulging into the left atrium, *jelly-like* in consistency, off-white, unevenly thickened with single small nodular thickenings ~3 mm in diameter. Mitral valve leaflets with growths of whitish dense tissue extending to the subvalvular endocardium. Chordal bands are whitish, some of them with local thickening up to 3 mm, some — thinned and elongated. Multiple additional chords are identified: longitudinal, diagonal and transverse, which in some areas create a net structure. The *aortic valve leaflets* are smooth, mobile, unevenly thickened mainly in the area of fibrous ring. In the area of transition to aortic bulb, yellowish non-protruding spots and white-yellow slightly protruding plaques were revealed.

Pulmonary valve leaflets are smooth, shiny, slightly unevenly thickened due to the connective tissue proliferation. The *papillary muscles are enlarged*, with growths of a whitish dense shiny tissue, especially pronounced in the LV. Some papillary muscles have a transverse attachment. In the LV cavity, mainly in the apex, there are additional trabeculae. Abnormal trabeculae are most pronounced in the apical, median-lateral and lower parts of the LV (Figure 4A, 4B). Between the trabeculae of both ventricles, as well as in the right atrial appendage, multiple dense dry gray-red *thrombotic masses are determined, which are difficult to separate from the endocardium* (Figure 4A).

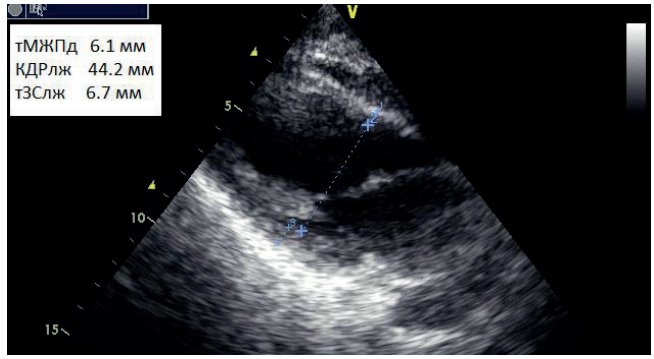


Figure 3A. Echocardiography of a 21-year-old female patient at the time of admission. Parasternal long axis view.
Abbreviations: LV EDD — left ventricular end-diastolic dimension, LVPWT — left ventricular posterior wall thickness, IVSTd — interventricular septal thickness at diastole.

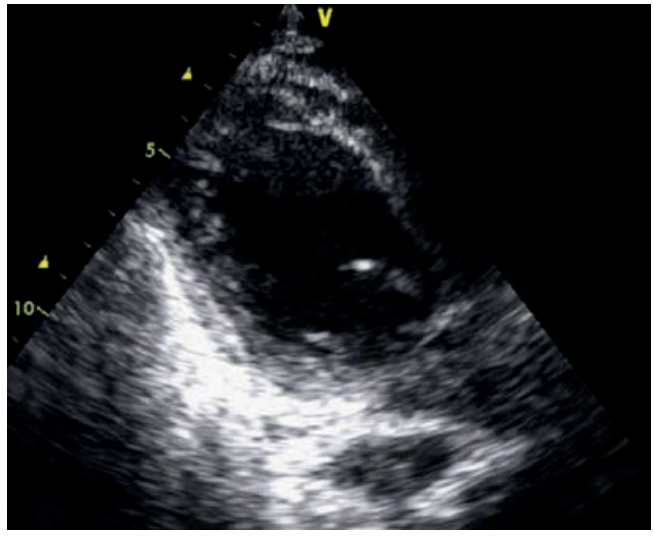


Figure 3B. Echocardiography, apical oblique view. In the area of LV apex and median segments of the inferior wall, there are additional trabeculae.

Red myocardium of a sloppy texture with diffuse growths of a whitish dense tissue and areas of violation of fibrous fine net-like structure. In the area of apex and interventricular septum (IVS), the mottled myocardial tissue with alternating yellowish and dark brown areas. In other areas, the myocardium is flabby with yellowish structureless layers. In the myocardium, the ratio of non-compact to compact layer was 3:1 (Figure 5A, B).

Coronary artery lumen was narrow throughout the entire length (up to 2 mm); the intima was yellow-gray, shiny with single lipid spots.

Right and left coronary arteries is narrowed due to circulated lipid deposits and fibrous plaques, narrowing the lumen of blood vessels by up to 50%. In the intramyocardial vessels, there are signs of acute discirculatory abnormalities and parietal thrombi. *Disorganization of collagen fibers, focal elastolysis, elastofibrosis in the arterial wall.* In the lumen of

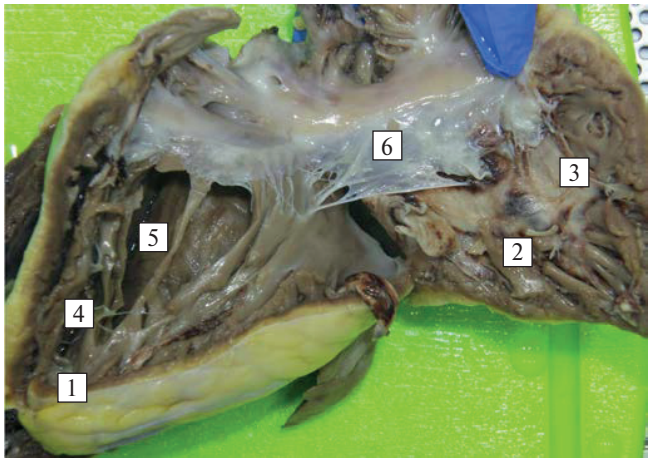


Figure 4A. Gross specimen. Pointed apex (1), subendocardial fibrosis (2), mixed parietal thrombus (3), additional chords (4), increased trabecularity (5). Indistinct structure of valvular endocardium (6).

Figure 4B. Gross specimen. Severe trabecularity and subendocardial motley structure.

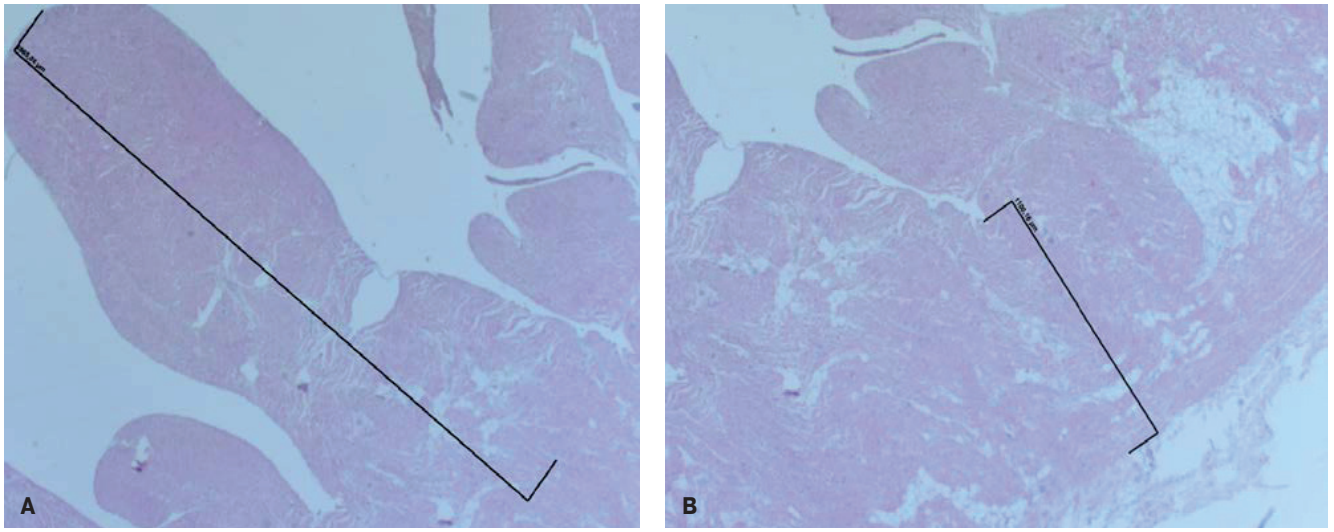


Figure 5A, B. Histological specimen of the myocardium: ratio of non-compact to compact layer, 3:1. Hematoxylin and eosin staining, x100.

small intramural vessels and distal parts of anterior interventricular and circumflex left coronary artery, dark red masses with a rough surface obturating vessels were revealed.

In the area of apex and IVS, myocardial tissue is variegated with alternating yellowish and dark brown areas. On the LV anterior wall with the transition to IVS, merging heterogeneous variegated foci are located. In this area, pale, yellowish, whitish and pink-red areas alternate. In the LV, diffuse and in places merging foci of ischemia and necrosis were found. In the cytogenic stroma with newly formed vessels, single lymphocytic accumulations are determined.

The total area of foci is ~28 cm^2. In the middle third of IVS, a focus of a similar type with an area of 10,5 cm^2 was found. There were areas of undulating deformation, fragmentation, dissociation, myocytolysis of muscle fibers, as well as large fields of fibrosis (Figure 6D).

Microscopic examination revealed changes in the left and right heart. The most pronounced abnormalities were detected in the LV myocardium with fields of rather normal muscle fibers and areas with lipomatosis, focal fibrosis, angiomatosis, among which there were groups of cardiomyocytes with polymorphic nuclei, foci of fragmentation and myocytolysis, areas of stromal basophilia (Figure 6A, 6C). In the right ventricle (RV), growths of adipose tissue were revealed. The residual area of cardiomyocytes in the RV in 1 sample was ~70%. The proportion of adipose tissue was 3%, and fibrous tissue — ~40%.

In the parietal and valvular endocardium, there were signs of connective tissue disorganization with a

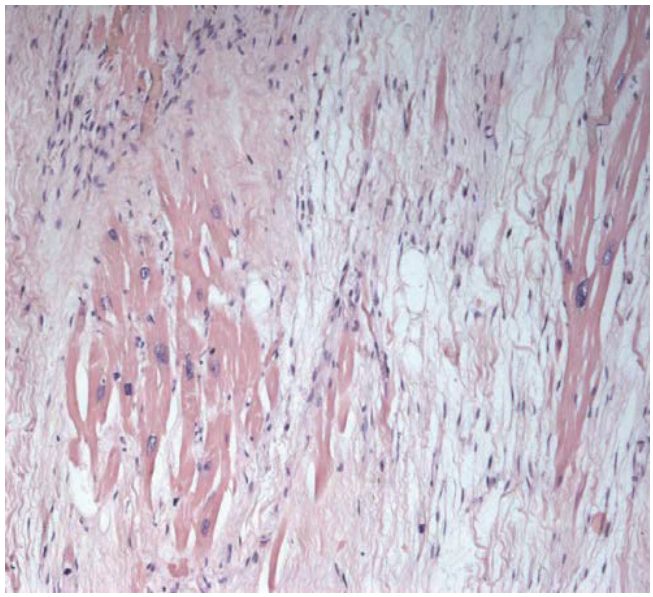


Figure 6A. LV myocardium: focal and interstitial fibrosis, angiomatosis and cardiomyocyte fields with polymorphic nuclei, foci of fragmentation and myocytolysis. Hematoxylin and eosin staining, x240.

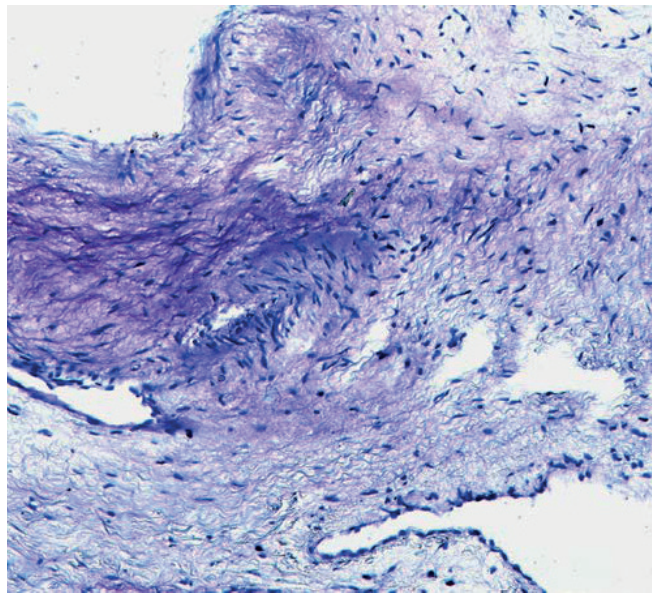


Figure 6B. Mitral valve: myxomatosis and superficial disorganization of connective tissue (mucoid swelling). Toluidine blue staining, x200.

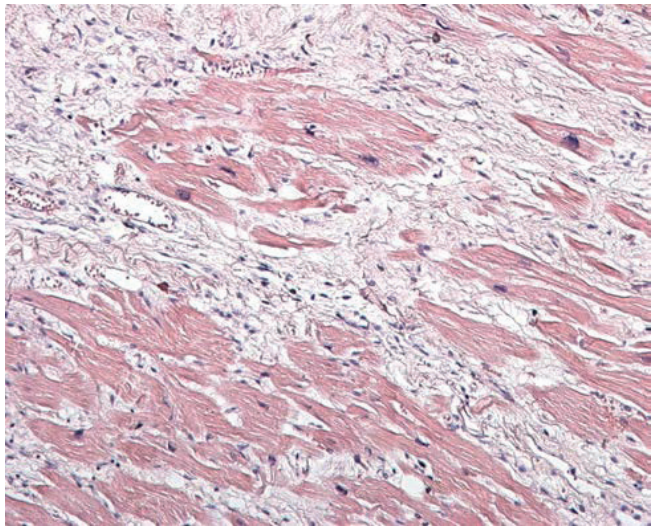


Figure 6C. LV myocardium: foci of the cellular stroma with basophilia. Hematoxylin and eosin staining, x240.

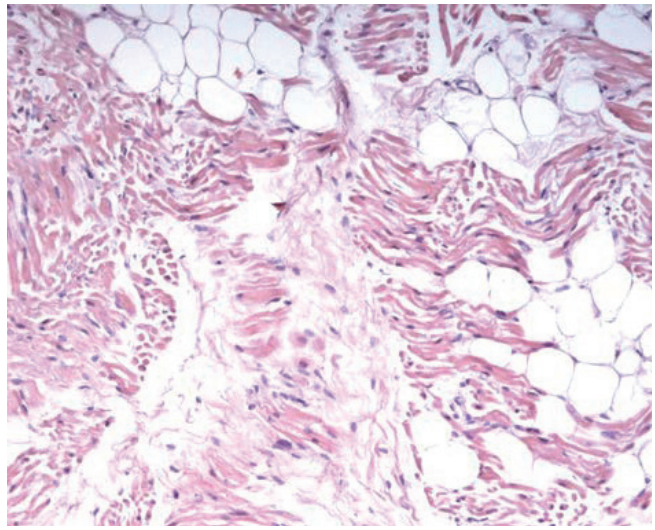


Figure 6D. RV myocardium: focal lipomatosis and undulating deformity of cardiomyocytes. Hematoxylin and eosin staining, x240.

predominance of mucoid swelling; in the heart valves, focal myxomatosis of varying severity was observed (Figure 6B). Growths of fibrous tissue were also found in the RV endocardium. In the myocardial stroma there were single (1-3) lymphocytes (Figure 6D).

Genetic analysis

To clarify the genetic nature of disease, a postmortem genetic testing was carried out using a new generation sequencing and Sanger sequencing with an Illumina MiSeq system.

A mutation in the *DSP* gene was found (uc021yle.1:exon23:c.C3300G:p.C1100W), which, according to the 2015 American College of Medical Genetics and Genomics (ACMG) classification, is with uncertain significance, however, taking into account the clinical performance can be causal [26].

A variant in the *KCNH2* gene (uc003wic.3:exon13:c.C3133T:p.L1045F) was also found, which, in accordance with the ClinVar database and ACMG classification, is considered as benign [26].

Thus, in young women:

- cardiac arrest was diagnosed against the background of electrical myocardial instability (VF);
- AF and long QT syndrome have been reported after cardiac arrest due to VF;

• echocardiography revealed additional trabeculae in the area of LV apex and inferior wall median segments;
• family history of SCD in two generations (unexplained death of the father under the age of 35 years);
• pathological examination revealed:
— hypertrabecularity/LV non-compaction;
— decrease in the cardiomyocyte area mainly in the RV myocardium, myocytolysis, foci of fibrosis and lipomatosis;
— parietal intracardiac blood clots; thrombi in intramyocardial vessels;
— coronary system anomalies;
— recurrent MI of various ages.

Discussion

Based on the comparison of macro- and microscopic abnormalities, it can be stated that the patient had NCC [27].

The clinical and morphological performance in this case also meets the criteria for ACM [6]. The initial morphological manifestations of ACM identified in the RV could also contribute to electrical cardiac instability and SCD.

Both ACM and NCC can manifest as symptoms and/or documented AF, conduction disorders, and tachyarrhythmias of RV and/or LV origin [28]. Repolarization abnormalities, recorded in patients with LVNC and in patients with ACM, also predispose to malignant ventricular tachyarrhythmias and SCD [27, 29]. The most typical repolarization disorders in this pathology are early repolarization (~40%) and long QT syndrome (50%) [30-32].

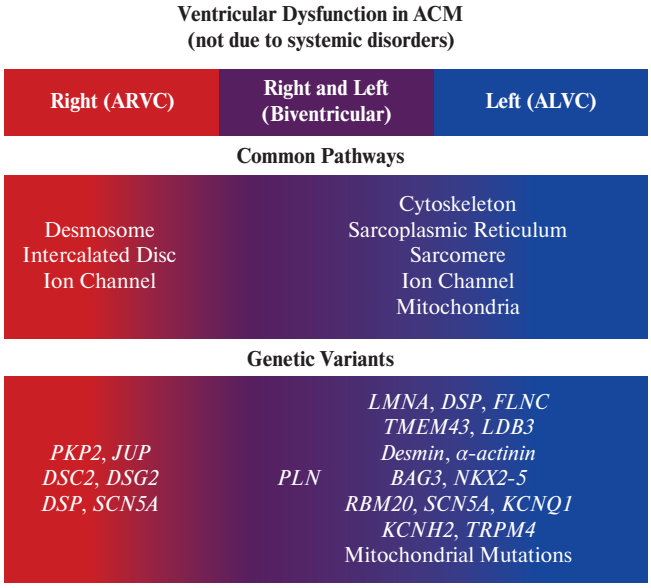


Figure 7. Approach to understanding the common pathway and genetic variants in a patient with arrhythmogenic cardiomyopathy (ACM) according to the predominant ventricular dysfunction [6]. **Abbreviations:** ALVC — arrhythmogenic left ventricular cardiomyopathy, ARVC — arrhythmogenic right ventricular cardiomyopathy.

The classic triad of *LVNC* complications includes: arrhythmias, including SCD, heart failure, systemic embolism. The disease can be asymptomatic for a long time [33-37].

The presence of intracardiac blood clots described in the patient may be an independent manifestation of NCC. Thromboembolic events occur in 5-38% of cases of LVNC [38]. It is extremely rare for NCC and the associated primary antiphospholipid syndrome, which in this case cannot be confirmed or ruled out [39, 40].

MI was clinically suspected (venous troponin I level 33,900 and than >50,000) and was confirmed by postmortem analysis. The literature describes combination of LVNC and subacute or acute type 1 MI of atherogenic origin in patients over 45 years of age [41-45].

Güvenç TS, et al. (2012) report a case of embolic MI in intact coronary arteries according to CA data in a 20-year-old man with LVMN [46]. Pulignano G, et al. Also described embolic MI in a 67-year-old woman with LVNC and LV thrombus. (2015) [47]. Despite this, the association of LVNC with coronary artery disease and MI is rare [48]. In this case, on the one hand, there is an abnormal structure of coronary system (narrow coronary arteries), which contributes to myocardial ischemia. On the other hand, the lumen of right and left coronary arteries was narrowed by up to 50% due to circular lipid spots and fibrous plaques. It cannot be ruled out that this fact may have clinical and hemodynamic significance.

Thus, LV lesions in this case are the result of a combination of non-coronary and coronary mechanisms. At the same time, this is, first of all, the result of chronic and acute dyscirculatory disorders in the intramyocardial arteries with creation of thrombi and thromboemboli.

Desmoplakin is a key element of desmosomes in cardiac and epithelial tissues, which plays the role of a structural component providing mechanical integrity and participates in a number of intracellular signaling pathways [49]. According to reviews, the association of *DSP* gene mutations and LVNC has been described by a number of authors [50-54]. Li S, et al. (2018) found that the development of LVNC among Chinese patients (n=100) is most often associated with the *TTN*, *MYH7, MYBPC3*, and *DSP* genes. The authors also emphasize that patients with LVNC and *DSP* genetic variants have a high risk of ventricular arrhythmias [55]. In the review by Arbustini E, et al. (2016), the *DSP* mutation is considered, manifesting as LVNC, LVNC and dilated CM, LVNC and asymptotic left ventricular dysfunction [50].

A current systemic analysis of genetic variants in ACM is presented in Towbin JA, et al. (2019) study (Figure 7) [6]. In 80% of cases of confirmed ACM, there are mutations in the genes of *PKP2, DSP*, and

DSG2 [56, 57]. Left-dominant ACM is associated with defects in *DSP* gene, as well as *KCNH2* and many other genes (Figure 7) [6, 58].

In our case, the described genetic variants may indicate not only the universal role of the structure of intercalated disk and desmosomes in CM origin, but also the existence of an unknown link that "directs" the CM to one or another morphofunctional phenotype [21].

Conclusion

The presented case report once again demonstrates that, regardless of the unique mechanism in embryonic heart development, the genetic spectrum of LVNC significantly overlaps with the other CMs and includes sarcomeric and structural genes, ion channel genes, and genes of intracellular metabolic pathways. Regardless of genetic nature of the disease, a family history of sudden death requires increased attention of clinicians to members of the proband's family for the early diagnosis of CM and prevention of SCD.

Relationships and Activities. This work was supported by the Russian Science Foundation grant 20-15-00271.

References

1. Berger S, Utech L, Fran Hazinski M. Sudden death in children and adolescents. Pediatric Clinics of North America. 2004;51(6 SPEC. ISS.):1653-77. doi:10.1016/j.pcl.2004.07.004.
2. Shlyakhto EV, Arutiunov GP, Belenkov YuN, et al. National guidelines for the risk stratification and the prevention of sudden cardiac death (second edition). M.: ID "Medpraktika-M"; 2018 p. 247. (In Russ.) ISBN: 978-5-98803-397-4.
3. Kaltman JR, Thompson PD, Lantos J, et al. Screening for sudden cardiac death in the young: Report from a national heart, lung, and blood institute working group. Circulation. 2011;123(17):1911-8. doi:10.1161/CIRCULATIONAHA.110.017228.
4. Maron BJ, Towbin JA, Thiene G, et al. Contemporary definitions and classification of the cardiomyopathies: An American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation. 2006;113(14):1807-16. doi:10.1161/CIRCULATIONAHA.106.174287.
5. Elliott P, Andersson B, Arbustini E, et al. Classification of the cardiomyopathies: A position statement from the european society of cardiology working group on myocardial and pericardial diseases. Eur Heart J. 2008;29(2):270-6. doi:10.1093/eurheartj/ehm342.
6. Towbin JA, McKenna WJ, Abrams DJ, et al. 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. Hear Rhythm. 2019;16(11):e301-72. doi:10.1016/j.hrthm.2019.05.007.
7. Corrado D, Wichter T, Link MS, et al. Treatment of arrhythmogenic right ventricular cardiomyopathy/dysplasia: An international task force consensus statement. Eur Heart J. 2015;36(46):3227-37. doi:10.1093/eurheartj/ehv162.
8. Smith W, Members of CSANZ Cardiovascular Genetics Working G. Guidelines for the Diagnosis and Management of Arrhythmogenic Right Ventricular Cardiomyopathy. Hear Lung Circ. 2011;20(12):757-60. doi:10.1016/j.hlc.2011.07.019.
9. Kapplinger JD, Landstrom AP, Salisbury BA, et al. Distinguishing arrhythmogenic right ventricular cardiomyopathy/dysplasia- associated mutations from background genetic noise. J Am Coll Cardiol. 2011;57(23):2317-27. doi:10.1016/j.jacc.2010.12.036.
10. Lombardi R, Marian AJ. Molecular genetics and pathogenesis of arrhythmogenic right ventricular cardiomyopathy: A disease of cardiac stem cells. Pediatr Cardiol. 2011;32(3):360-5. doi:10.1007/s00246-011-9890-2.
11. Lutokhina YuA, Blagova OV, Nedostup AV, et al. Clinical types (classification) of the right ventricle arrhythmogenic dysplasia: specifics of diagnostics and management. Russ J Cardiol. 2018;(2):19-31. (In Russ.) doi:10.15829/1560-4071-2018-2-19-31.
12. Thiene G, Nava A, Corrado D, et al. Right Ventricular Cardiomyopathy and Sudden Death in Young People. N Engl J Med. 1988;318(3):129-33. doi:10.1056/NEJM198801213180301.
13. Gordeeva MV, Mitrofanova LB, Pakhomov AV, et al. Arrhythmogenic right ventricular cardiomyopathy/dysplasia as a cause of sudden cardiac death of young adults. J Arrhythmology. 2012;(69):38-48. (In Russ.)
14. Fontaine G, Fontaliran F, Frank R. Arrhythmogenic right ventricular cardiomyopathies: Clinical forms and main differential diagnoses. Circulation. 1998;97(16):1532-5. doi:10.1161/01.CIR.97.16.1532.
15. Fontaine G, Brestescher C, Fontaliran F, et al. Outcome of arrhythmogenic right ventricular dysplasia. Apropos of 4 cases. Arch Mal Coeur Vaiss. 1995;88(7):973-9.
16. Arbustini E, Narula N, Dec GW, et al. The MOGE(S) classification for a phenotype-genotype nomenclature of cardiomyopathy: Endorsed by the world heart federation. J Am Coll Cardiol. 2013;62(22):2046-72. doi:10.1016/j.jacc.2013.08.1644.
17. Aras D, Ozeke O, Cay S, et al. Arrhythmogenic noncompaction cardiomyopathy: Is there an echocardiographic phenotypic overlap of two distinct cardiomyopathies? J Cardiovasc Ultrasound. 2015;23(3):186-90. doi:10.4250/jcu.2015.23.3.186.
18. Poliakova A, Krutikov A, Semernin E, et al. The clinical use of the MOGE(S) classification in the differential diagnosis between idiopathic hypertrophic cardiomyopathy and its phenocopies. Russian Journal of Cardiology. 2019;24(11):35-41. (In Russ.) doi:10.15829/1560-4071-2019-11-35-41.
19. Melnik OV, Gudkova AY, Vershinina TL, et al. Clinical polymorphism of RASopathies in terms of the children's cardiology department. Cons Medicum. 2017;19(12):100-4. (In Russ.) doi:10.26442/2075-1753_19.12.100-104.
20. Miszalski-Jamka K, Jefferies JL, Mazur W, et al. Novel Genetic Triggers and Genotype-Phenotype Correlations in Patients with Left Ventricular Noncompaction. Circ Cardiovasc Genet. 2017;10(4):e001763. doi:10.1161/CIRCGENETICS.117.001763.
21. Vaikhanskaya TG, Sivitskaya LN, Kurushko TV, et al. Left ventricular noncompaction: a distinct cardiomyopathy or a composite anatomical syndrome? Kardiologiia. 2018;58(11S):33-45. (In Russ.) doi:10.18087/cardio.2558.
22. Galaktionov AA. Troponin: reference range for blood tests, kinds of tests, causes of its elevation in myocardial infarction. (In Russ.) https://cardiogid.com/troponin/. (24 Aug 2020).
23. Pacheco Claudio C, Quesada O, Pepine CJ, et al. Why names matter for women: MINOCA/INOCA (myocardial infarction/ischemia and no obstructive coronary artery disease). Clin Cardiol. 2018;41(2):185-93. doi:10.1002/clc.22894.
24. Averkov OV, Barbarash OL, Boytsov SA, et al. Differentiated approach in diagnostics, diagnosis formulation, case management and statistical accounting of type 2 myocardial infarction (Position Paper). Russ J Cardiol. 2019;(6):7-21. (In Russ.) doi:10.15829/1560-4071-2019-6-7-21.
25. Scalone G, Niccoli G, Crea F. Editor's Choice- Pathophysiology, diagnosis and management of MINOCA: an update. Eur Hear journal Acute Cardiovasc care. 2019;8(1):54-62. doi:10.1177/2048872618782414.
26. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24. doi:10.1038/gim.2015.30.
27. Udeoji DU, Philip KJ, Morrissey RP, et al. Left ventricular noncompaction cardiomyopathy: Updated review. Ther Adv Cardiovasc Dis. 2013;7(5):260-73. doi:10.1177/1753944713504639.
28. Marcus FI, McKenna WJ, Sherrill D, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/Dysplasia: Proposed modification of the task force criteria. Circulation. 2010;121(13):1533-41. doi:10.1161/CIRCULATIONAHA.108.840827.
29. Mavrogeni SI, Bacopoulou F, Apostolaki D, et al. Sudden cardiac death in athletes and the value of cardiovascular magnetic resonance. Eur J Clin Invest. 2018;48(7):e12955. doi:10.1111/eci.12955.
30. Miyake CY, Kim JJ. Arrhythmias in Left Ventricular Noncompaction. Cardiac Electrophysiology Clinics. 2015;7(2):319-30. doi:10.1016/j.ccep.2015.03.007.
31. Caliskan K, Ujvari B, Bauernfeind T, et al. The prevalence of early repolarization in patients with noncompaction cardiomyopathy presenting with malignant ventricular arrhythmias. J Cardiovasc Electrophysiol. 2012;23(9):938-44. doi:10.1111/j.1540-8167.2012.02325.x.
32. Zhou H, Lin X, Fang L, et al. Prolonged QTc indicates the clinical severity and poor prognosis in patients with isolated left ventricular non-compaction. Int J Cardiovasc Imaging. 2017;33(12):2013-20. doi:10.1007/s10554-017-1209-9.
33. Lahmiti S, Aboussad A. Isolated non-compaction of the right ventricular myocardium: two cases report. Ann Cardiol d'Angeiol. 2012;61(4):299-302. doi:10.1016/j.ancard.2010.12.001.
34. Stöllberger C, Gerecke B, Finsterer J, Engberding R. Refinement of echocardiographic criteria for left ventricular noncompaction. Int J Cardiol. 2013;165(3):463-7. doi:10.1016/j.ijcard.2011.08.845.

35. Ichida F, Hamamichi Y, Miyawaki T, et al. Clinical features of isolated noncompaction of the ventricular myocardium: Long-term clinical course, hemodynamic properties, and genetic background. J Am Coll Cardiol. 1999;34(1):233-40. doi:10.1016/S0735-1097(99)00170-9.
36. Nugent AW, Daubeney PEF, Chondros P, et al. The epidemiology of childhood cardiomyopathy in Australia. N Engl J Med. 2003;348(17):1639-46. doi:10.1056/NEJMoa021737.
37. Lilje C, Rázek V, Joyce JJ, et al. Complications of non-compaction of the left ventricular myocardium in a paediatric population: A prospective study. Eur Heart J. 2006;27(15):1855-60. doi:10.1093/eurheartj/ehl112.
38. Paterick TE, Jamil Tajik A. Left ventricular noncompaction: A diagnostically challenging cardiomyopathy. Circulation Journal. 2012;76(7):1556-62. doi:10.1253/circj.CJ-12-0666.
39. Pugliatti P, Di Bella G, Recupero A, et al. Non compaction cardiomyopathy and Antiphospholipid syndrome: A catastrophic thromboembolic association. Int J Cardiol. 2008;128(1):126-8. doi:10.1016/j.ijcard.2007.05.043.
40. Tenedios F, Erkan D, Lockshin MD. Cardiac Manifestations in the Antiphospholipid Syndrome. Rheumatic Disease Clinics of North America. 2006;32(3):491-507. doi:10.1016/j.rdc.2006.05.008.
41. Gabrielli FA, Lombardo A, Natale L, et al. Myocardial infarction in isolated ventricular non-compaction: Contrast echo and MRI. Int J Cardiol. 2006;111(2):315-7. doi:10.1016/j.ijcard.2005.09.054.
42. Swinkels BM, Boersma LVA, Rensing BJ, et al. Isolated left ventricular noncompaction in a patient presenting with a subacute myocardial infarction. Netherlands Hear J. 2007;15(3):109-11. doi:10.1007/BF03085964.
43. Correia AF, Oliveira DC, Sanctos M. Coronary Artery Thromboses, Stent Thrombosis and Antiphospholipid Antibody Syndrome: Case Report. Cardiol Res. 2018;9(2):129-32.
44. Toufan M, Shahvalizadeh R, Khalili M. Myocardial infarction in a patient with left ventricular noncompaction: A case report. Int J Gen Med. 2012;(5):661-5. doi:10.2147/IJGM.S28902.
45. Fettouhi H, Tamdy A, Ellouali F, et al. Convulsives crisis revealing left-ventricular non-compaction with apical myocardial infarction. Ann Cardiol Angeiol. 2011;60(3):159-64. doi:10.1016/j.ancard.2010.12.008.
46. Güvenç TS, Erer HB, Altay S, et al. "Idiopathic" acute myocardial infarction in a young patient with noncompaction cardiomyopathy. Cardiol J. 2012;19(4):429-33. doi:10.5603/CJ.2012.0077.
47. Pulignano G, Tinti MD, Tolone S, et al. Noncompaction and embolic myocardial infarction: The importance of oral anticoagulation. Rev Port Cardiol. 2015;34(7-8):497.e1-497.e4. doi:10.1016/j.repc.2015.01.014.
48. Panduranga P, Mukhaini MK. Left-ventricular non-compaction with coronary artery disease. Int J Cardiol. 2011;150(1):e37-e39. doi:10.1016/j.ijcard.2009.09.476.
49. Martherus R, Jain R, Takagi K, et al. Accelerated cardiac remodeling in desmoplakin transgenic mice in response to endurance exercise is associated with perturbed wnt/β-catenin signaling. Am J Physiol — Hear Circ Physiol. 2016;310(2):H174-87. doi:10.1152/ajpheart.00295.2015.
50. Arbustini E, Favalli V, Narula N, et al. Left Ventricular Noncompaction: A Distinct Genetic Cardiomyopathy? Journal of the American College of Cardiology. 2016;68(9):949-66. doi:10.1016/j.jacc.2016.05.096.
51. Williams T, Machann W, Kühler L, et al. Novel desmoplakin mutation: juvenile biventricular cardiomyopathy with left ventricular non-compaction and acantholytic palmoplantar keratoderma. Clin Res Cardiol. 2011;100(12):1087-93. doi:10.1007/s00392-011-0345-9.
52. Tufekcioglu O, Aras D, Sahin O, et al. Two cardiomyopathies in one heart. Echocardiography. 2006;23(6):519-21. doi:10.1111/j.1540-8175.2006.00253.x.
53. Wlodarska EK, Wozniak O, Konka M, et al. Isolated ventricular non-compaction mimicking arrhythmogenic right ventricular cardiomyopathy — A study of nine patients. Int J Cardiol. 2010;145(1):107-11. doi:10.1016/j.ijcard.2009.05.062.
54. Streltsova AA, Gudkova AY, Kostareva AA. Left ventricular non-compaction: contemporary view of genetic background, clinical course, diagnostic and treatment.Terapevticheskii Arkhiv. 2019;91(12):90-7. (In Russ.) doi:10.26442/00403660.2019.12.000142.
55. Li S, Zhang C, Liu N, et al. Genotype-positive status is associated with poor prognoses in patients with left ventricular noncompaction cardiomyopathy. J Am Heart Assoc. 2018;7(20):e009910. doi:10.1161/JAHA.118.009910.
56. Towbin JA. Inherited cardiomyopathies. Circulation Journal. 2014;78(10):2347-56. doi:10.1253/circj.CJ-14-0893.
57. Herren T, Gerber PA, Duru F. Arrhythmogenic right ventricular cardiomyopathy/dysplasia: A not so rare "disease of the desmosome" with multiple clinical presentations. Clinical Research in Cardiology. 2009;98(3):141-58. doi:10.1007/s00392-009-0751-4.
58. Sen-Chowdhry S, Syrris P, Prasad SK, et al. Left-Dominant Arrhythmogenic Cardiomyopathy. An Under-Recognized Clinical Entity. J Am Coll Cardiol. 2008;52(25):2175-87. doi:10.1016/j.jacc.2008.09.019.

https://russjcardiol.elpub.ru
doi:10.15829/1560-4071-2020-4115

ISSN 1560-4071 (print)
ISSN 2618-7620 (online)

The combination of left ventricular non-compaction and hypertrophic cardiomyopathy in one family with a pathogenic variant in the *MYBPC3* gene (rs397516037)

Myasnikov R. P.[1], Kulikova O. V.[1], Meshkov A. N.[1], Mershina E. A.[2], Kiseleva A. V.[1], Klimushina M. V.[1], Divashuk M. G.[1,3], Kurilova O. V.[1], Pilyus P. S.[2], Kharlap M. S.[1], Koretsky S. N.[1], Larina O. M.[2], Sinitsyn V. E.[2], Gandaeva L. A.[4], Barsky V. I.[4], Basargina E. N.[4], Boytsov S. A.[5], Drapkina O. M.[1]

The article presents the results of clinical, instrumental and molecular genetic tests of three generations of a family with inherited cardiomyopathy caused by a new variant in the *MYBPC3* gene. A specific feature of this case is the phenotypic heterogeneity of the mutation — a combination of hypertrophic cardiomyopathy and left ventricular non-compaction in family members. Attention is drawn to the various severity of clinical manifestations in relatives of carriers of mutation: from asymptomatic to severe heart failure and acute cerebrovascular accident.

Key words: left ventricular non-compaction, hypertrophic cardiomyopathy, heart failure, sudden cardiac death, thromboembolism, acute cerebrovascular accident, *MYBPC3*.

Relationships and Activities: none.

[1]National Medical Research Center for Therapy and Preventive Medicine, Moscow; [2]Medical Research and Education Center, Lomonosov Moscow State University, Moscow; [3]All-Russian Research Institute of Agricultural Biotechnology, Kurchatov Genomics Center, Moscow; [4]National Medical Research Center for Children's Health, Moscow; [5]National Medical Research Center of Cardiology, Moscow, Russia.

Myasnikov R. P. ORCID: 0000-0002-9024-5364, Kulikova O. V.* ORCID: 0000-0002-3138-054X, Meshkov A. N. ORCID: 0000-0001-5989-6233, Mershina E. A. ORCID: 0000-0002-1266-4926, Kiseleva A. V. ORCID: 0000-0003-4765-8021, Klimushina M. V. ORCID: 0000-0002-7876-9325, Divashuk M. G. ORCID: 0000-0001-6221-3659, Kurilova O. V. ORCID: 0000-0003-3082-5161, Pilyus P. S. ORCID: 0000-0001-7802-2734, Kharlap M. S. ORCID: 0000-0002-6855-4857, Koretsky S. N. ORCID: 0000-0001-6009-5775, Larina O. M. ORCID: 0000-0002-2484-5249, Sinitsyn V. E. ORCID: 0000-0002-5649-2193, Gandaeva L. A. ORCID: 0000-0003-0890-7849, Barsky V. I. ORCID: 0000-0003-1267-1517, Basargina E. N. ORCID: 0000-0002-0144-2885, Boytsov S. A. ORCID: 0000-0001-6998-8406, Drapkina O. M. ORCID: 0000-0002-4453-8430.

*Corresponding author:
olgakulikova2014@mail.ru

Received: 21.09.2020
Revision Received: 30.09.2020
Accepted: 09.10.2020

For citation: Myasnikov R. P., Kulikova O. V., Meshkov A. N., Mershina E. A., Kiseleva A. V., Klimushina M. V., Divashuk M. G., Kurilova O. V., Pilyus P. S., Kharlap M. S., Koretsky S. N., Larina O. M., Sinitsyn V. E., Gandaeva L. A., Barsky V. I., Basargina E. N., Boytsov S. A., Drapkina O. M. The combination of left ventricular non-compaction and hypertrophic cardiomyopathy in one family with a pathogenic variant in the *MYBPC3* gene (rs397516037). *Russian Journal of Cardiology*. 2020;25(10):4115. (In Russ.) doi:10.15829/1560-4071-2020-4115

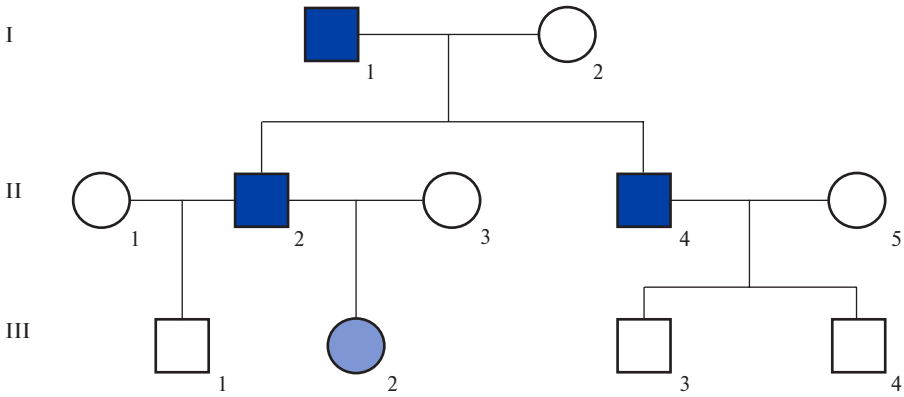
Left ventricle noncompaction (LVNC) is characterized by a two-layered myocardial structure with a thin, compacted outer (epicardial) band and a much thicker, non-compacted inner (endomyocardial) layer and deep myocardial trabeculae, particularly in the apex and free wall of the left ventricle. The clinical performance of the disease is extremely diverse, but the following symptoms usually predominate: heart failure (HF), cardiac arrhythmias and thromboembolic events. These disorders can be either sporadic or familial. Of particular interest is familial LVNC. Currently, genes have been identified that lead to LVNC. The largest number of mutations associated with this disease are localized in sarcomere protein genes. This category also includes the *MYBPC3* gene, a mutation in which is most often associated with hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). In this article, we present a family with *MYBPC3* gene mutation and different types of cardiomyopathy in generations.

The aim of our study was to demonstrate a familial case of cardiomyopathies of different phenotypes.

Material and methods

On the basis of a multicenter registry of LVNC patients, a family (Figure 1) with a familial LVNC in combination with HCM was selected. All participants signed informed consent. The study design was approved by the ethics committee of the National Medical Research Center for Therapy and Preventive Medicine (Moscow, Russia). All participants underwent diagnostic tests according to the protocol described earlier [1]. The LVNC was established on the basis of echocardiography and magnetic resonance imaging (MRI) [2, 3].

DNA isolation was performed using a QIAamp DNA Blood Mini Kit (Qiagen, Germany). The DNA concentration was determined on a Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA). Next-generation sequencing was performed on a Nextseq550 system (Illumina, USA). Whole-



Number	Diagnosis	Mutation in the *MYBPC3* gene
I-1	57 years old. HCM. First-degree AV block. Second-degree AV block. VT. NYHA class II, stage 2A HF. ICD implantation.	+
I-2	57 years old, healthy. Increased LV trabecularity.	-
II-1	30 years old, healthy.	no data
II-2	34 years. Combination of hypertrophic and dilated type of LVNC. NYHA class IIA, stage 2A HF. Pulmonary hypertension. Lysed thrombus in the LV apex. ICD implantation.	+
II-3	35 years old, healthy.	no data
II-4	36 years. Combination of hypertrophic and dilated type of LVNC. NYHA class IIA, stage 2A HF. Stroke.	+
II-5	Not known.	no data
III-1	2 years old, healthy.	-
III-2	11 years. Increased LV trabecularity.	+
III-3	5 years old, not examined.	+
III-4	2 years, not examined.	-

Figure 1. Family lineage.
Abbreviations: AV — atrioventricular, HCM — hypertrophic cardiomyopathy, VT — ventricular tachycardia, ICD — cardioverter defibrillator, LV — left ventricle, LVNC — left ventricular noncompaction, HF — heart failure.

Table 1

Echocardiography parameters

№	LVNC criteria			EDV	IVST	EF
	Stollberger	Jenni	Chin			
I-1	-	-	+	121	2,5	69%
I-2	+	-	-	75	0,8	61%
II-2	-	+	+	150	1,6	41%
II-4	+	+	+	225	2,0	48%
III-2	-	+	-	51	0,5	70%

Abbreviations: EDV — end-diastolic volume, IVST — interventricular septal thickness, EF — ejection fraction.

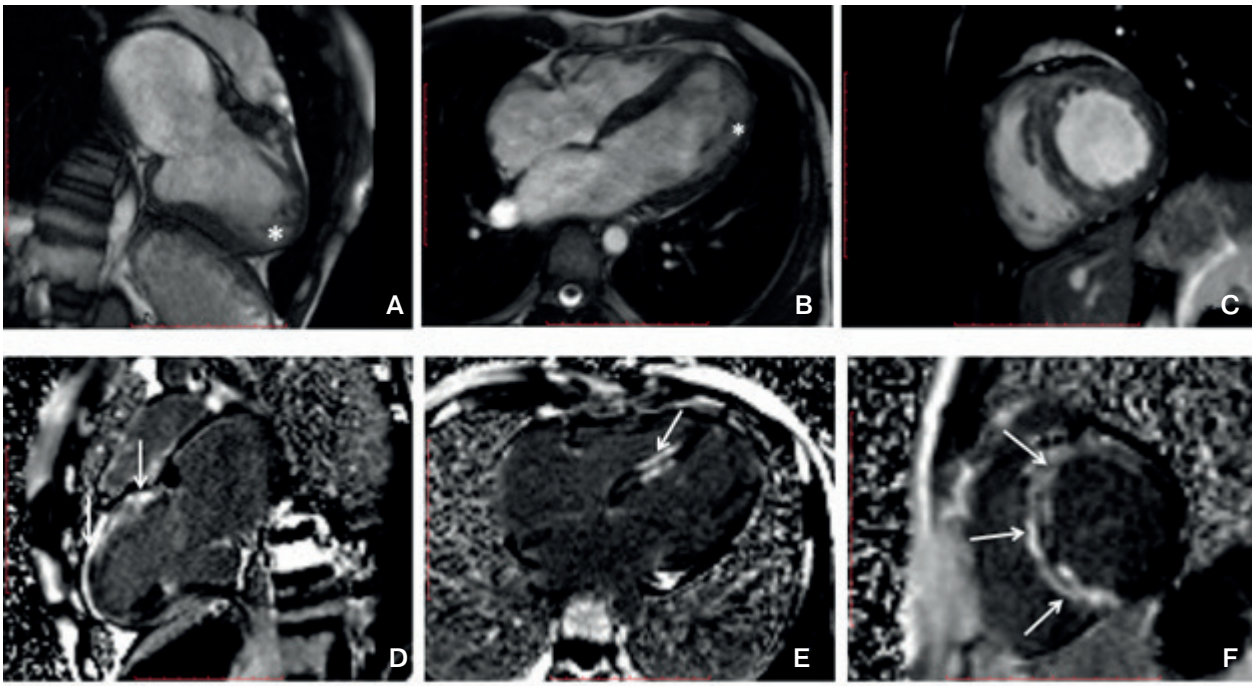


Figure 2. (**A**-**C**) Cardiac MRI of the proband, SSFP sequence: **A** — long axis 2-chamber view, **B** — long axis 4-chamber view, **C** — short axis.
Note: * — NC layer (**D**-**F**) — delayed contrast enhancement, IR sequence with suppression of the myocardial signal. Arrows indicate extended areas of subepicardial and intramyocardial contrast enhancement in the middle septal and anterior segments.

exome sequencing was performed using IDT-Illumina TruSeq DNA Exome (Illumina, USA). As a result of sequencing and bioinformatics analysis, .fastq and .vcf files were obtained. For clinical interpretation, genetic variants with frequencies in the gnomAD database <0,5% were selected. The assessment of the pathogenicity was carried out in accordance with the ACMG/AMP 2015 guidelines. The identified variants were validated by Sanger sequencing. The nucleotide sequence of PCR products was determined using the ABI PRISM® BigDye™ Terminator v.3.1 kit with subsequent analysis of the reaction products on an Applied Biosystem 3500 DNA Analyzer (Thermo Fisher Scientific, USA).

Results

The 34-year-old proband was cared due to HCM from childhood. At the age of 20 years, LV ejection fraction (EF) was 78%, interventricular septum (IVS) thickness — 15 mm. After this, the patient was not observed for a long time and did not take therapy. At 31, he noted an increase in shortness of breath and the development of edema, which required hospital treatment. Echocardiography revealed LVEF of 35-40%, pulmonary artery systolic pressure of 60 mm Hg, hypokinesis of the apex and LV anteroseptal wall, a mobile thrombus in the LV apex. Chest multislice computed tomography revealed a LV thrombus, bilateral hydrothorax. No data for pulmonary embolism were obtained. With HF therapy with anticoagulants,

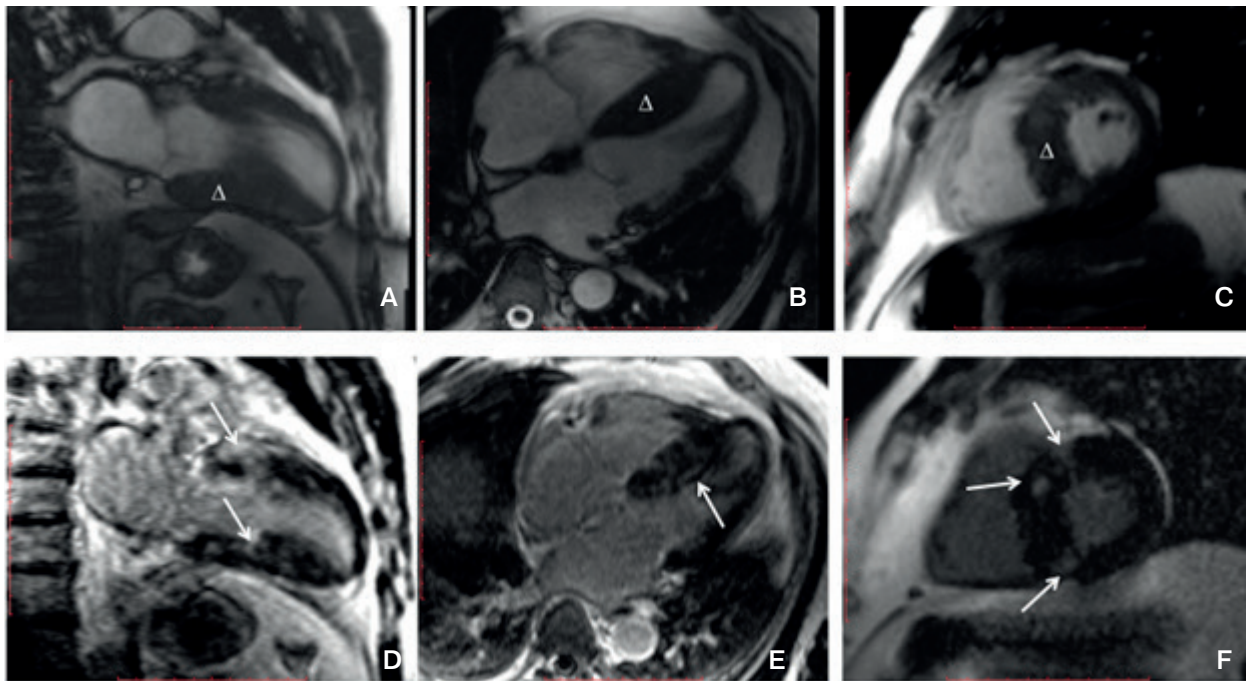


Figure 3. (**A**-**C**) Cardiac MRI of the proband's father, SSFP sequence: **A** — long axis 2-chamber view, **B** — long axis 4-chamber view, **C** — short axis, (**D**-**F**) — delayed contrast enhancement.
Note: Δ — asymmetric hypertrophy of the IVS myocardium and LV inferior wall, IR sequence with suppression of the myocardial signal. Arrows indicate intramyocardial foci of contrasting in hypertrophied segments.

HF symptoms regressed and LV thrombus was lysed. Echocardiography revealed left atrium of 5,4 cm, end diastolic volume of 150 ml, IVS thickness of 1,4-1,9 cm. In the region of the apex, lateral wall, posterior wall of the LV, signs of noncompaction (Jenni, Stollberger criteria) was detected. We also revealed a LV ejection fraction of 41%, hypokinesis of the anteroseptal wall with involvement of the apex, pulmonary artery systolic pressure of 44 mm Hg (Table 1). Contrast-enhanced cardiac MRI identified IVS hypertrophy up to 16 mm, signs of noncompaction of the apex, lateral wall, and posterior wall of the LV (Figure 2). The thickness of compacted layer in these segments was 6 mm, and the non-compacted layer — 18 mm. The noncompaction mass index was 20 g/m^2 (20% of the LV mass). LVEF was 48%. Pronounced LV myocardial fibrosis was noted.

According to 24-hour Holter monitoring, paroxysmal ventricular tachycardia (VT) were recorded (maximum, 7 complexes). Given the high risk of sudden cardiac death (SCD), a dual-chamber cardioverter-defibrillator (ICD) was implanted. Despite the regular use of prescribed therapy of HF with beta-blockers (BB), angiotensin II receptor blockers and neprilysin inhibitors, mineralocorticoid receptor antagonists (MCRA), loop diuretics, the patient has annual hospitalizations due to decompensated HF. Given the pronounced LVM, a history of LV apex thrombosis, the presence of systolic dysfunction, in order to prevent thromboembolism, the patient was prescribed vitamin K antagonists. After this, there were no signs of recurrent intracardiac thrombosis according to echocardiography.

The 57-years-old proband's father. At the age of 35, the HCM was verified during a routine examination. At the same time, he noted rare cardiac interruptions. No drug therapy was prescribed. From the age of 50, he began to notice the shortness of breath with a gradual decrease in exercise tolerance. At the age of 54, due to his son's illness, he was examined at the clinic. Echocardiography revealed normal contractility, asymmetric LV hypertrophy, IVS fibrosis, moderately pronounced LVNC in the apex and lateral wall. LV false chords and LVEF of 69% was established. According to the 24-hour Holter monitoring, first-degree atrioventricular (AV), second-degree transient AV block with 16 pauses up to a maximum of 4 seconds, 1542 ventricular premature beats, 5 episodes of VT was detected. Contrast-enhanced cardiac MRI (Figure 3) revealed an asymmetric non-obstructive biventricular HCM, intramyocardial fibrosis, and LVEF of 74%. The noncompaction mass index was 10 g/m^2. No congestion in the systemic circulation was observed, but the level of pro-brain natriuretic peptide was 833 pg/ml. Given the high risk of SCD, a dual-chamber ICD was implanted for the primary prevention of SCD. After discharge from

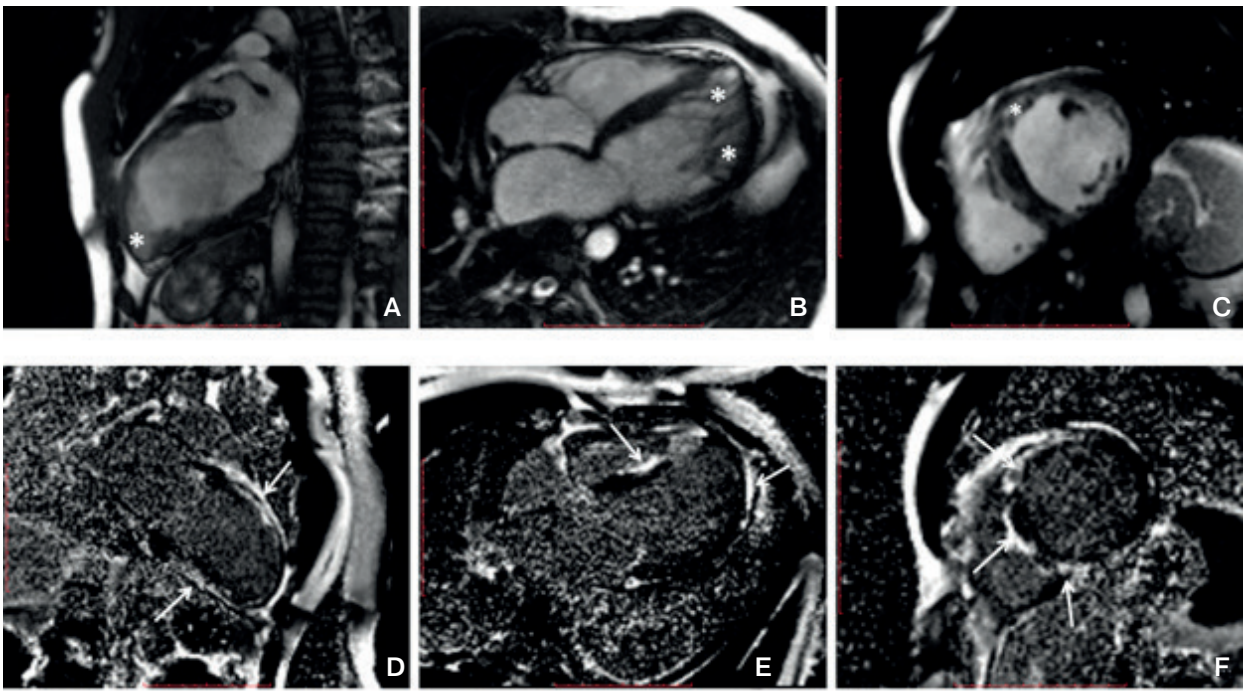


Figure 4. (**A**-**C**) Cardiac MRI of the proband's brother, SSFP sequence: **A** — long axis 2-chamber view, **B** — long axis 4-chamber view, **C** — short axis.
Note: * — NC layer (**D**-**F**) — delayed contrast enhancement, IR sequence with suppression of the myocardial signal. Arrows indicate extended areas of subepicardial and intramyocardial contrast in the middle septal, anterior and lower segments.

the hospital, he regularly takes BB, angiotensin-converting enzyme (ACE) inhibitors, MCRA, loop diuretics, against which the heart failure was compensated and stable episodes of paroxysmal VT were not recorded.

The 36-year-old proband's brother, which in childhood was cared simultaneously with his brother due to HCM. He did not take any medications, and was not further examined. Due to brother's illness, he was examined at the clinic. According to the 24-hour Holter monitoring, there was a rare ventricular premature beats and no VT runs. Echocardiography revealed a LV dilatation. End diastolic dimension was 6,4 cm, EF — 48%, IVS thickness — 20 mm. There were signs of noncompaction of the apex, lateral and posterior walls of the LV. He did not take the recommended therapy with BB, ACE inhibitors, MCRA. Contrast-enhanced cardiac MRI (Figure 4) 18 months after the echocardiography established IVS hypertrophy up to 14 mm, signs of noncompaction of the apex, anterior, lateral and posterior walls of the LV. The noncompaction mass index was 24 g/m^2, which is 22% of the LV mass. LVEF was 35%. Fibrosis of the IVS, the anterior, inferior and apical segments of the LV was revealed. HF therapy with BB, ACE inhibitors, MCRA, and also, taking into account the systolic dysfunction and a pronounced LVNC, vitamin K antagonists was recommended. The patient did not take the recommended therapy. Later, left middle cerebral artery (MCA) stroke developed.

The 57-year-old proband's mother was examined for LVNC with other family members. Echocardiography showed nondilated cardiac chambers, normal LV systolic function, and increased trabecularity of the apex and lateral wall of the LV, corresponding to the noncompaction criteria (Stollberger). Cardiac MRI did not reveal LVNC (Figure 5), while a significant number of additional LV false chords were visualized.

Cardiac screening examination of 11-year-old proband's daughter was performed. Echocardiography showed nondilated cardiac chambers, normal IVS, and increased trabecularity of the LV apex. According to cardiac MRI, increased LV trabecularity did not meet criteria for LVNC (Figure 6).

Genetic analysis. The proband had a heterozygous variant rs397516037 in exon 33 of the *MYBPC3* gene (hg19: chr11: 47353740) NM_000256.3:c.3697C>T, which led to the stop codon NP_000247.2:p.Gln1233Ter. This variant was detected in proband (II-2), in the proband's father (I-1), brother (II-4), daughter (III-2), and nephew (III-3). It was not detected in the proband's mother (I-2), son (III-1) and second nephew (III-4).

Discussion

C-MYBPC3 (cardiac myosin-binding protein-C) is arrayed transversely in sarcomere A-bands and

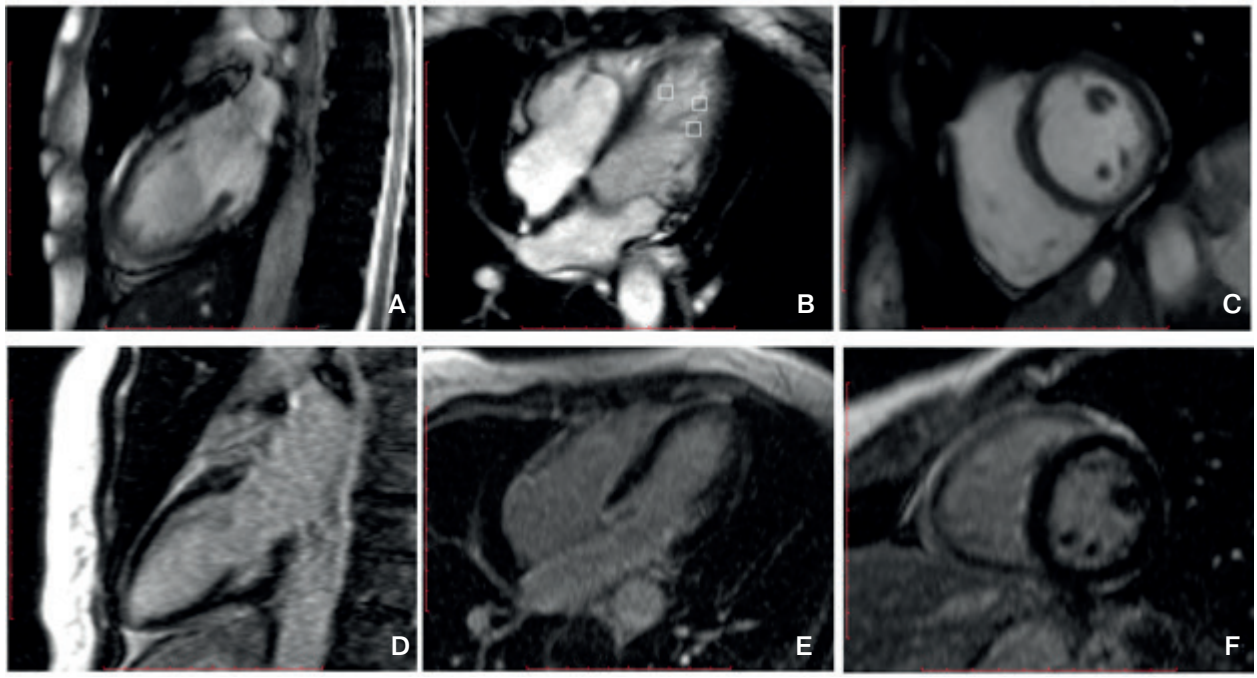


Figure 5. (**A**-**C**) Cardiac MRI of the proband's mother, SSFP sequence: **A** — long axis 2-chamber view, **B** — long axis 4-chamber view, **C** — short axis, (**D**-**F**) — delayed contrast enhancement.
Note: □ — additional LV chords, IR sequence with suppression of the myocardial signal. There were no areas of intramyocardial contrasting.

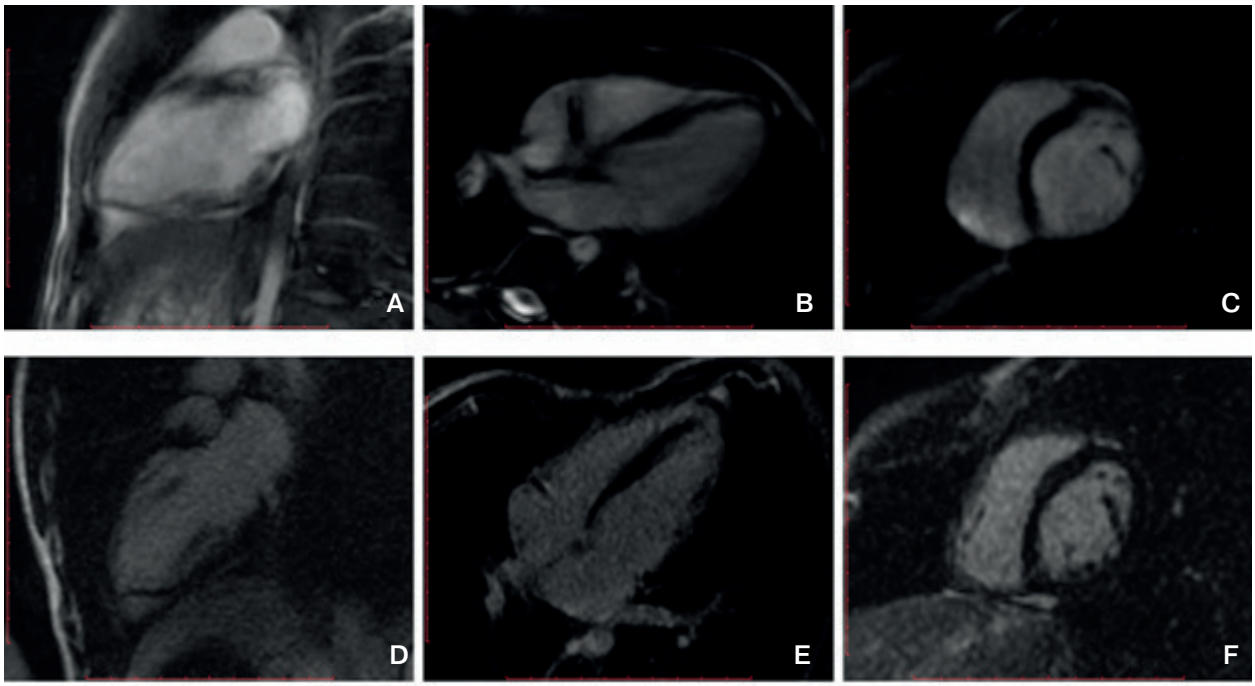


Figure 6. (**A**-**C**) Cardiac MRI of the proband's daughter, SSFP sequence: **A** — long axis 2-chamber view, **B** — long axis 4-chamber view, **C** — short axis, cardiac chambers are not enlarged, not hypertrophied, contractility is not reduced, (**D**-**F**) — delayed contrasting IR sequence with suppression of the myocardial signal. There were no areas of intramyocardial contrasting.

binds myosin heavy chain in thick filaments and titin in elastic filaments [4]. Previs MJ, et al. found that C-MYBPC3 slows actomyosin motion generation in native cardiac thick filaments. This mechanical effect was localized to where cMyBP-C resides within the thick filament (i.e., the C-zones) and was modulated by phosphorylation and site-specific proteolytic degradation. The authors concluded that

Table 2

Cardiac MRI parameters

№	EDV, ml/m^2	LVEF, %	Grothoff				Jacquier, %	Petersen
			NC Mass index, g/m^2	NC mass-to-total mass ratio, %	Noncompacted-to-compacted myocardial mass ratio ⩾3:1 in one segment 1-3, 7-16	Noncompacted-to-compacted myocardial mass ratio ⩾2:1 in 4-6 segments		
I-1	55	74	10	12	-	-	12	-
I-2	61	60	6	12	-	-	12	-
II-2	100	48	20	20	+	-	20	+
II-4	123	35	24	22	+	+	22	+
III-2	73	57	14	20	-	-	20	-

Abbreviations: EDV — end diastolic volume, NC — noncompaction, LVEF — left ventricular ejection fraction.

Table 3

Cardiac MRI data by segments

№	Noncompacted-to-compacted myocardial mass ratio by segments																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
I-1	0,0	0,0	0,0	0,0	1,1	0,9	0,8	0,0	0,0	0,0	1,9	0,9	0,0	0,0	0,0	1,0	0,0
I-2	1,5	0,0	0,0	0,0	0,6	0,0	2,4	0,0	0,0	2,3	1,7	0,0	0,0	0,0	0,0	3,0	0,0
II-2	1,4	2,3	0,0	1,0	1,0	1,2	2,5	0,7	1,5	2,0	2,2	3,0	1,1	0,0	0,8	1,6	2,8
II-4	2,7	1,0	0,0	2,1	1,3	2,0	3,7	0,0	0,0	1,4	2,9	3,8	3,6	0,0	3,4	1,1	3,3
III-2	0,0	0,0	0,0	0,0	0,0	0,0	1,5	0,0	0,0	0,0	0,0	0,0	1,8	0,0	0,0	0,0	0,0

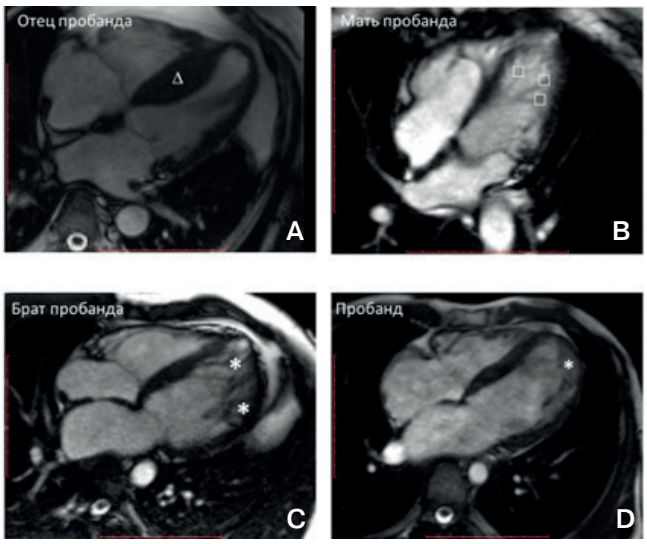


Figure 7. (**A**-**D**) Cardiac MRI, SSFP sequence, long axis 4-chamber view.
Note: Δ — hypertrophied segments of the myocardium, * — noncompact layer of the myocardium, □ — additional LV chords.

cMyBP-C should be considered a member of a tripartite complex with actin and myosin that allows fine tuning of cardiac muscle contraction [5].

A mutation in the *MYBPC3* gene was first described in patients with HCM in 1995 [6]. Subsequently, mutations in this gene have also been described in DCM and LVNC [7, 8].

In 2017, the study by Sedaghat-Hamedani F, et al. was published, which demonstrated the mutation in the *MYBPC3* gene in a large family of several generations with diagnosed LVNC [9]. The authors also asked how different alleles in the same gene can lead to significantly different phenotypes of the disease. Thus, a pathogenic variant in the *MYH7* gene was identified, which was described as HCM, but the proband's phenotype in this study was LVNC. In the study by Richard, et al., 95 patients with LVNC were examined and they underwent exome sequencing, according to the results of which possible genetic causes were found in 50% of cases and a mutation in the *MYBPC3* gene was detected in only 4% of cases [10].

In the study by Waning JI, et al. in 2019, 172 papers were analyzed and the correlation of genotypes and phenotypes of LVNC was carried out [11]. Both variants in the *MYBPC3* and *TTN* genes were associated with cardiac events and had a poorer prognosis. Also in 2019, the same group of authors presented data from work on familial LVNC [12]. All patients were divided into 4 types of LVNC: dilated, hypertrophic, isolated NC, combination of dilated and hypertrophic types. A comparative analysis of

remodeling types in LVNC showed that hypertrophic type had a more favorable prognosis compared to othyrs. It is important to note that the *MYBPC3* gene mutation aggravated the disease course and also led to a poor prognosis.

The presented case of familial LVNC confirms the above facts of the phenotypic heterogeneity of myocardial remodeling in the presence of *MYBPC3* gene mutation, as well as a more unfavorable prognosis in patients with signs of LV dilatation.

In one family, we see that the proband's father has nonobstructive HCM, the proband and his sibling have IVS hypertrophy and signs of LVNC. The proband's daughter has increased trabeculariry without criteria for LVNC, HCM and LV cavity dilatation (Figure 7). The most pronounced degree of LVNC in the proband's brother: 3 Grothoff criteria for LVNC, noncompaction mass of 24 g/m^2; the Grothoff criterion for the relative mass of LVNC in both brothers is slightly below the threshold value (25%) due to the pronounced IVS hypertrophy (Table 2). In addition, the number of segments with LVNC in the proband's brother is significantly greater than in the proband himself (Table 3). Also noteworthy is the presence of massive myocardial fibrosis in the father and brothers as a possible cause of VT, while the proband's brother has fibrosis in almost all LV segments (Figure 4). The disease course in brothers with LVNC is significantly more severe than in the father with HCM. The proband has progressive heart failure with a high risk of SCD, and the proband's brother has severe consequences of stroke as an element of thromboembolic events in LVNC. It should be noted that the proband's brother refused to take HF therapy and anticoagulants.

Despite the absence of clinical manifestations, the 11-year-old proband's daughter needs close attention and dynamic observation, since the presence of morphological changes that are currently slightly manifested, as well as mutations in the *MYBPC3* gene, may lead to the formation of pathological changes in the myocardium (fibrosis, IVS hypertrophy).

Conclusion

Phenotypic heterogeneity of mutations in genes associated with cardiomyopathies both as manifestations of HCM, DCM and LVNC in members of the same family, and as a combination of various types of myocardial remodeling in LVMC, dissolve the boundaries between pathogenetic and etiological aspects of genetically determined cardiomyopathies. At the same time, the presence of LVNC undoubtedly requires additional measures of therapy and prevention.

Relationships and Activities: none.

References

1. Myasnikov RP, Shcherbakova NV, Kulikova OV, et al. Des gene mutation in a family of proband with myofibrillary myopathy and non-compaction cardiomyopathy, resulted in cardiac transplantation. Russian Journal of Cardiology. 2017;(10):9-16. (In Russ.) doi:10.15829/1560-4071-2017-10-9-16.
2. Petersen SE, Selvanayagam JB, Wiesmann F, et al. Left Ventricular Non-Compaction. Journal of the American College of Cardiology. 2005;46(1):101-5. doi:10.1016/j.jacc.2005.03.045.
3. Jacquier A, Thuny F, Jop B, et al. Measurement of trabeculated left ventricular mass using cardiac magnetic resonance imaging in the diagnosis of left ventricular non-compaction. European Heart Journal. 2010;31(9):1098-104. doi:10.1093/eurheartj/ehp595.
4. Luther PK, Winkler H, Taylor K, et al. Direct visualization of myosin-binding protein C bridging myosin and actin filaments in intact muscle. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(28):11423-8. doi:10.1073/pnas.1103216108.
5. Previs MJ, Beck Previs S, Gulick J, et al. Molecular mechanics of cardiac myosin-binding protein C in native thick filaments. Science. 2012;337(6099):1215-8. doi:10.1126/science.1223602.
6. Watkins H, Conner D, Thierfelder L, et al. Mutations in the cardiac myosin binding protein–C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. Nature Genetics. 1995;11(4):434-7. doi:10.1038/ng1295-434.
7. Daehmlow S, Erdmann J, Knueppel T, et al. Novel mutations in sarcomeric protein genes in dilated cardiomyopathy. Biochemical and Biophysical Research Communications. 2002;298(1):116-20. doi:10.1016/S0006-291X(02)02374-4.
8. Probst S, Oechslin E, Schuler P, et al. Sarcomere gene mutations in isolated left ventricular noncompaction cardiomyopathy do not predict clinical phenotype. Circulation Cardiovascular genetics. 2011;4(4):367-74. doi:10.1161/CIRCGENETICS.110.959270.
9. Sedaghat-Hamedani F, Haas J, Zhu F, et al. Clinical genetics and outcome of left ventricular non-compaction cardiomyopathy. European Heart Journal. 2017;38(46):3449-60. doi:10.1093/eurheartj/ehx545.
10. Richard P, Ader F, Roux M, et al. Targeted panel sequencing in adult patients with left ventricular non-compaction reveals a large genetic heterogeneity. Clinical Genetics. 2019;95(3):356-67. doi:10.1111/cge.13484.
11. van Waning JI, Moesker J, Heijsman D, et al. Systematic Review of Genotype-Phenotype Correlations in Noncompaction Cardiomyopathy. Journal of the American Heart Association. 2019;8(23):e012993. doi:10.1161/JAHA.119.012993.
12. van Waning JI, Caliskan K, Michels M, et al. Cardiac Phenotypes, Genetics, and Risks in Familial Noncompaction Cardiomyopathy. Journal of the American College of Cardiology. 2019;73(13):1601-11. doi:10.1016/j.jacc.2018.12.085.